ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/326227471

Biofertilizers: A Sustainable Approach for Pulse Production

Chapter · July 2018

DOI: 10.1007/978-981-13-0253-4_14

CITATIONS
0

READS
683

2 authors, including:

Anup Das
Indian Council of Agricultural Research
180 PUBLICATIONS **1,020** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Resource conservation and crop diversification in rice/maize based cropping systems for enhancing climate resilience View project

National Mission on Sustaining Himalayan Ecosystem, Task Force 6 View project

All content following this page was uploaded by Anup Das on 03 August 2018.

The user has requested enhancement of the downloaded file.

Biofertilizers: A Sustainable Approach for Pulse Production

14

Subrata Nath Bhowmik and Anup Das

Contents

14.1 Introduction 446
14.1.1 What Are Biofertilizers? 447
14.2 Beneficial Microbes and Pulse Production 448
14.2.1 Microorganisms for Nitrogen Fixation 449
14.2.2 Multiple Microorganisms for Enhancing Pulse Productivity 457
14.3 Formulations of Biofertilizers for Sustainable Agriculture 463
14.3.1 Inoculant Formulations 464
14.3.2 AMF Inoculants 469
14.3.3 Mixed Bacterial Inoculants 470
14.3.4 Formulations for Microbial Consortia: Inoculants for the Future 471
14.4 Future Perspectives of Biofertilizers in Pulse Production 472
14.5 Conclusion 474
References 474

Abstract

Nutrient needs of plants can be met through a number of sources which include mineral fertilizers, organic manures, recycled wastes and by-products, biological nitrogen (N) fixation (BNF), natural minerals and to lesser extent nutrients recycled through irrigation waters, and precipitation. These supplement the soil nutrient reserves for nourishing the crops. Presently, soil management strategies are mainly dependent on inorganic chemical-based fertilizers, which caused a serious threat to human health and environment. The exploitation of beneficial microbes as a biofertilizer has become a paramount importance in agriculture for their potential role in food security and sustainable productivity. The eco-friendly

S. Nath Bhowmik (✉)
ICAR Research Complex for NEH Region, Tripura Centre, Lembucherra, Tripura, India

A. Das
Division of Crop Production, ICAR Research Complex for NEH Region, Umiam, India

© Springer Nature Singapore Pte Ltd. 2018

R. S. Meena et al. (eds.), *Legumes for Soil Health and Sustainable Management*,
https://doi.org/10.1007/978-981-13-0253-4_14

approaches inspire a wide range of application of plant growth-promoting rhizobacteria (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria, and many other useful microscopic organisms. The interactions of these beneficial microbes with environment determine crop health in natural agroecosystem by providing numerous services to crop plants, viz., soil organic matter (SOM) decomposition, nutrient acquisition and recycling, weed control, water absorption, and biocontrol, thus enhancing soil fertility and maintaining soil heath in eco-friendly manner. Various complementing combinations of microbial inoculants for management of major nutrients such as N and phosphorus (P) are necessary for sustainable production. Biofertilizers also cut the cost of chemical fertilizers used in agriculture considerably. An estimated amount of US$ 1421–15,237 of chemical fertilizer in the form of urea per hectare per year can be substituted by biofertilizer. The present chapter highlights the broad canvas of biofertilizers that enhance N and P nutrition in varied crops with special reference to pulses in the form of several perspectives. The mode of action of these microorganisms within and the transformation of nutrients elucidated. In the Indian scenario, the use of biofertilizers faces various constraints, such as longevity, etc. that need to be overcome to achieve substantial fertilizer savings. One of the key issues that remain is the method of formulation of these biofertilizers. Some prospective solutions to tackle the issue are brought out in this chapter.

Keywords
Biofertilizer · Biological nitrogen fixation · N-fixing microorganism · Biological nitrogen fixation · Phosphate-solubilizing microorganism · PGPR

14.1 Introduction

The modern agriculture played a pivotal role in meeting the food demands of a burgeoning human population, which has also led to an escalating reliance on synthetic agrochemicals like fertilizers and pesticides (Santos et al. 2012). Synthetic fertilizers are industrially manufactured substances composed of known quantities of macronutrients (e.g., N, P, potassium (K), etc.) and micronutrients (e.g., zinc (Zn), boron (B), iron (Fe), etc.), and their indiscriminate and imbalance use causes air, ground, and water pollution by nitrate (NO3) leaching and surface water bodies pollution through eutrophication (Youssef and Eissa 2014). Considering this, recent efforts have been focused toward the production of "nutrient-rich high-quality food" to ensure biosafety (Bhardwaj et al. 2014; Buragohain et al. 2017). The innovative approach of farm production attracts the increasing demand of biological fertilizers as a viable option to agrochemicals (Raja 2013). Biological fertilizers or biofertilizers enrich the soil environment with micro- and macronutrients via N-fixation and P and K solubilization or mineralization and enhance plant growth-regulating/growth-promoting substances, production of antibiotics, and biodegradation of organic matter (OM) in the soil (Sinha et al. 2014).

14.1.1 What Are Biofertilizers?

Biofertilizers are carrier or liquid-based preparations containing microorganisms, in sufficient numbers which benefit plant growth and nutrition (Motsara et al. 1995). Depending upon their roles and association with plants, biofertilizer are of a different kind (Fig. 14.1). The use of biofertilizer can reduce the N, P, and K requirement of crops from chemical fertilizer sources. They are largely acknowledged to be low-cost supplements to chemical fertilizers and devoid of harmful effect either on soil health or environment. The additional advantages of biofertilizers include longer shelf life of microbial cells causing no adverse effects to the ecosystem (Sahoo et al. 2014). When biofertilizers are used as seed or soil inoculants, they multiply and participate in nutrient cycling and contribute to crop growth and productivity (Motsara et al. 1995). In general, 60–90% of the total applied fertilizer is lost, and the remaining 10–40% is absorbed by plants (Bhardwaj et al. 2014). Thus, microbial inoculants have a high significance in integrated nutrient management systems (INMS) for sustaining agricultural productivity and promoting healthy environment (Adesemoye and Kloepper 2009).

India is the largest producer, consumer, and importer of pulses in the world, with 24% share in the global production (Reddy 2009). The major pulse crops grown in India are *Cicer arietinum*, *Cajanus cajan*, *Vigna radiata*, *Vigna mungo*, *Lens culinaris*, and *Pisum sativum* (Reddy and Reddy 2010). About 90% of the global *Cajanus*

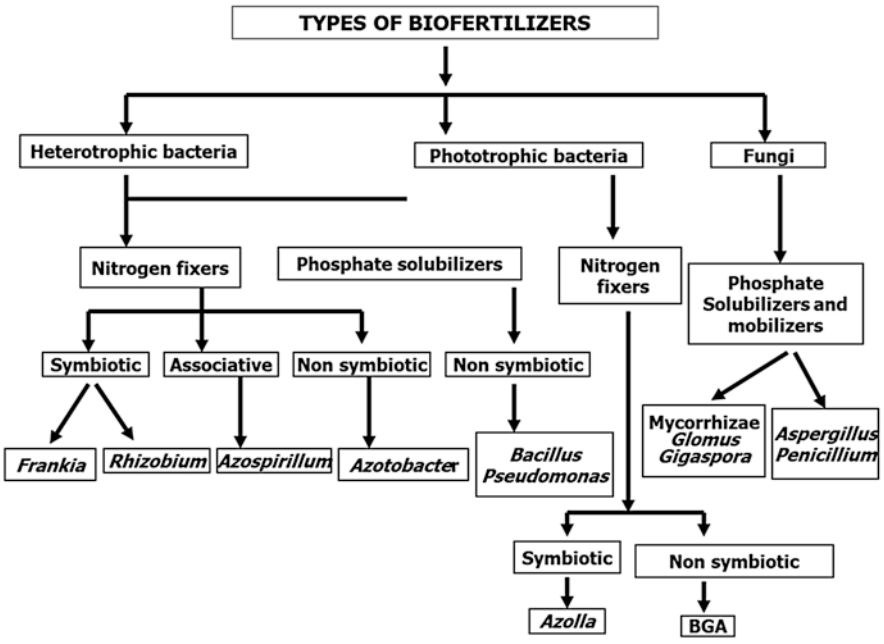

Fig. 14.1 Types of biofertilizer and their functions (Motsara et al. 1995)

cajan, 65% of *Cicer arietinum*, and 37% of *Lens culinaris* area fall in India, corresponding to 93%, 68%, and 32% of the global production, respectively (FAOSTAT 2009). The country's pulses production has been hovering around 14–15 million tones (Mt) from a near-stagnated area of 22–23 Mha, since 1990–1991 (Ali and Gupta 2012; Meena et al. 2015a). The country imports pulses ~2.5 to 3.5 Mt every year to meet the protein requirement of the nation. The strongly increasing trend in the import of pulses is a cause of concern, since an increase in demand from India has a cascading effect on international prices, thus putting tremendous pressure on the precious foreign exchange (Reddy 2009). By 2050, the domestic requirements of pulses would be ~26.50 Mt, demanding substantial stepping up in the production (Reddy 2004). This challenging task has to be accomplished under increasing production constraints, especially abiotic stresses, abrupt climatic changes, the emergence of new species/strains of insect pests and diseases, and multiple deficiencies of secondary and micronutrients in the soil (Ali and Gupta 2012).

This chapter is intended to cater to the needs of agriculturists and plant biologists whose work focuses in eco-friendly field management that reveals the potential application of biofertilizers for sustainable crop culture with special reference to pulse production. Further, the mode of actions of biofertilizers within and the transformation of nutrients elucidated. The use of biofertilizers faces various constraints, such as longevity, viable cell counts, etc. that need to be overcome to achieve substantial fertilizer savings. One of the key issues that remain is the method of formulation of these biofertilizers. Prospects and some of the key difficulties associated with the use of biofertilizers in sustainable agriculture with special reference to pulses are brought out in this chapter.

14.2 Beneficial Microbes and Pulse Production

Rhizosphere, the narrow zone of soil surrounding plant roots, contains ~1011 microbial cells per gram of root and >30,000 prokaryotic species that in general improve plant growth and productivity (Egamberdieva et al. 2008; Mendes et al. 2013). The collective genome of rhizosphere microbial community is larger compared to that of plants and is referred to as microbiome (Bulgarelli et al. 2013) whose interactions determine crop health in natural agroecosystem through numerous services being provided to crop plants, viz., nutrient acquisition, OM decomposition, nutrient recycling, water absorption, and pest control (Berg et al. 2013). Rhizosphere microbial communities as an option for synthetic fertilizers have become a subject of importance in sustainable agriculture and biosafety program. The agriculturally useful microbial populations encompass plant growth-promoting rhizobacteria (PGPR), N-fixing cyanobacteria, mycorrhiza, plant disease suppressive beneficial bacteria, stress-tolerant endophytes, and biodegrading microbes (Singh et al. 2011; Meena et al. 2015b). The term PGPR is currently applied to a wide spectrum of strains that have, in common, the ability to promote plant growth following inoculation onto seeds and subterranean plant parts (Kloepper et al. 1988; Bhowmik and Singh 2004).

14.2.1 Microorganisms for Nitrogen Fixation

For optimum pulses productivity, N is an essential plant nutrient (Dudeja et al. 2011). Since it is widely consumed by the majority of plants, most of the soils are deficient in it. Moreover, soil N is also lost due to leaching and volatilization (Brahmaprakash and Sahu 2012). Although air contains 78.09% N, plants cannot make use of it as such. The N-fixing bacteria synonymously called diazotrophs are a special type of microorganisms which can reduce atmospheric N into ammonia in the presence of nitrogenase enzyme. Microorganisms and plants assimilate N in their body parts in ammonical form for growth and development. On the basis of their mode of N-fixation, these bacteria are classified into three physiological groups, i.e., symbiotic, associative symbiotic, and free living (Fig. 14.2).

14.2.1.1 Chemical V/S Biological Nitrogen Fixation

In most of the agricultural systems, N is often the most limiting nutrient that dictates crop production. Despite its presence in large quantities in the atmosphere, plants cannot utilize N since it is in an inert form (Brahmaprakash and Sahu 2012). N is made available in the form of fertilizers which are chemical fixation of atmospheric N through the Haber-Bosch process (Motsara et al. 1995). This process requires high temperature (400–500 °C) and pressure (20 Mpa) and corresponds to energy inputs of about 875 m^3 of natural gas, 5.5 barrels of oil, or 2 mt of coal to fix one metric ton of ammonia (Dixon and Wheeler 1986). Dinitrogen is the most stable

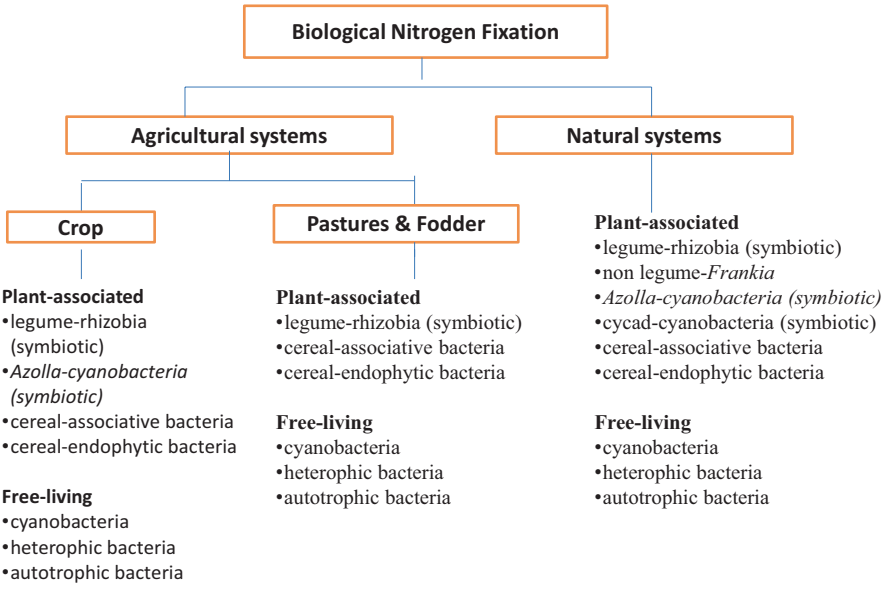

Fig. 14.2 Biological nitrogen-fixing agents in agricultural and terrestrial natural systems (Modified: Herridge et al. 2008)

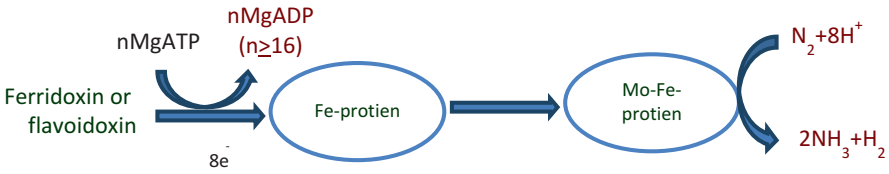

Fig. 14.3 Reduction of dinitrogen to ammonia by nitrogenase enzyme complex

diatomic molecule known, and two atoms are joined by a very stable triple bond. Very high amount of energy (945 kJ) is required to break this triple bond and therein rests one of the major challenges of dinitrogen fixation (Herridge et al. 2008).

Atmospheric dinitrogen can also be fixed biologically (by diazotrophs = *prokaryotes* that fix dinitrogen) to ammonia, which is utilized by plants. Prokaryotes are unicellular organisms lacking a well-defined nucleus (Stanier 1987). The ammonia may also be converted to nitrate by few microorganisms in the soil which is then available to plant (Madigan et al. 2009). The nitrate thus formed is amenable for denitrification reactions in deeper horizons of soil leading to the formation of N_2 gas which will escape to the atmosphere. This is the typical path of N cycle.

BNF is bacteria-mediated enzymatic fixation of N at ambient temperature and pressure (Pelczar et al. 1993). The magnitude of BNF in the biosphere is not easy to determine, but approximately it amounts to ~107 Mt/year compared to ~160 Mt/year of man-made N-fixation which is 1.5 times higher than the natural fixation (Galloway et al. 2008). In the global N cycle, every N atom in the atmosphere cycles once in a million year (Postgate 1989). BNF contributes 65% of N consumption in agriculture (Burris and Roberts 1993). All the bacteria fixing atmospheric N catalyze the reaction through nitrogenase enzyme. The nitrogenase enzyme has two components – (1) Mo-Fe protein, called dinitrogenase, and (2) Fe protein, called dinitrogenase reductase. First Mo-Fe protein takes part in reducing dinitrogen to ammonia, and second Fe protein assists Mo-Fe protein by providing electrons for reduction of dinitrogen. The mechanism of N fixation is the same in all N-fixing bacteria; the reduction of one molecule of dinitrogen requires 16 ATP in vitro and 20–30 ATP under field conditions, as it is less efficient (Fig. 14.3).

14.2.1.2 Symbiotic Nitrogen Fixation

Legume-*Rhizobium* symbiosis is an important aspect of symbiotic nitrogen fixation (SNF) which is optimally exploited to benefit agriculture for sustainability. Over a century ago, German scientists, Hellriegel and Wilfarth, experimentally demonstrated the N-fixation in legume nodule by nodule-inducing ferment (*Rhizobium*): the stage was set for the popularity of the *Rhizobium* inoculation technology world over. In this symbiosis, macro-symbiont is the legume plant, and micro-symbiont is the prokaryotic bacteria *(Rhizobium).* The macro-symbiont legume belongs to the Leguminosae family, divided into three subfamilies comprising of 700 genera and 14,000 species (Beringer et al. 1979). Only about 200 of these are cultivated by man.

Table 14.1 Cross-inoculation group and *Rhizobium*-legume association (Morel et al. 2012)

Rhizobia	Legume cross-inoculation group
Bradyrhizobium japonicum	Soybean group: *Glycine max*
Ensifer meliloti	Alfalfa group: *Melilotus* spp., *Trigonella* spp., *Medicago sativa*,
Rhizobium leguminosarum bv *trifolii*	Clover group (clover I, II, III, and IV): *Trifolium* spp.
Bradyrhizobium spp.	Cowpea group: *Arachis hypogea*, *Cajanus cajan*, *Vigna radiata*, *Vigna mungo*, *Vigna unguiculata*, *Vigna* spp., *Phaseolus lunatus*, *Acacia mearnsii, Enterolobium* spp., *A. mangium, Desmodium* spp., *Psophocarpus tetragonolobus*, *Albizia* spp., *Stylosanthes* spp., *Centrosema* sp., *Voandzeia subterranea*, *Lablab purpureus*, *Cyamopsis tetragonoloba*, *Calopogonium mucunoides*, *Pueraria phaseoloides*, *Macroptilium atropurpureum*
Rhizobium leguminosarum bv *phaseoli*	Bean group: *Phaseolus coccineus*, *Phaseolus vulgaris*
Rhizobium leguminosarum bv *viciae*	Pea group: *Pisum* spp., *Vicia* spp., *Vicia faba*, *Lens culinaris*
Rhizobium lupine	Group lupines
Mesorhizobium loti	Chickpea group: *Lotus corniculatus* L., *Cicer* spp.,
Rhizobium spp.	Crown vetch

Rhizobium is symbiotic bacterium which fixes atmospheric N in leguminous and certain nonlegumes like *Parasponia* plants by forming nodules (Bhardwaj et al. 2014; Meena et al. 2017a). Pink-colored nodules are considered most effective in N-fixation varying from 50 to 200 kg N/ha per season (Motsara et al. 1995). It is helpful for pulse legumes like *L. culinaris*, *C. arietinum*, *C. cajan*, *V. radiata*, *V. mungo*, *P. sativum*, etc., oil-seed legumes like *Glycine max* and *Arachis hypogea*, forage legumes like *Trifolium* and *Medicago sativa*, and tree legumes like *Leucaena*. *Rhizobium*, a symbiotic and crop-specific bio-inoculant, can be used for legume crop and trees, for example, *Rhizobium meliloti* for *Medicago sativa*, *Rhizobium trifolii* for *Trifolium*, *Rhizobium phaseoli* for temperate beans, *Rhizobium japonica* for *Glycine max*, *Rhizobium leguminosarum* for *Lens culinaris* and *Pisum sativum*, and *Rhizobium lupini* for *Cicer arietinum*. Hence, the use of right inoculant for the right legume is the farmer key for obtaining adequate benefit from the use of *Rhizobium* biofertilizers. Such information must be given by the manufacturer and clearly specified on the label. Plants mutually compatible with the same species of rhizobia are called "cross-inoculation groups" (Table 14.1). With regard to rhizobia, a considerable change in taxonomic status has come about during recent times. Sahgal and Johri (2003) outlined the current rhizobial taxonomy and enlisted 36 species distributed among 7 genera (*Azorhizobium*, *Allorhizobium*, *Mesorhizobium*, *Methylobacterium*, *Bradyrhizobium*, *Rhizobium*, and *Sinorhizobium*), based on the polyphasic taxonomic approach. *Rhizobium* is however limited by their specificity, and only certain legumes are benefited by this symbiosis. There is a need to develop an efficient symbiosis of host-specific rhizobial isolates and also to develop isolates with superior nodulation competitiveness that can overcome the limitations of low N-fixation, poor crop yield, and poor effectiveness under field conditions.

The genus *Mesorhizobium* has been described as in between *Rhizobium* and *Bradyrhizobium* and identified in Asia, Africa, Europe, Australia, South and North America, and the Arctic (Jarvis et al. 1997; Poinsot et al. 2001). Different species of mesorhizobia such as *M. temperadae*, *M. mediterraneum*, *M. tianshanense*, and *M. ciceri* have been reported to form nodules in *C. arietinum* (Dudeja and Narula 2008).

Rhizobium inoculation increases the crop yield in the range 10–35% (Motsara et al. 1995). However, variable crop responses to *Rhizobium* inoculation in the main field were noticed in the regime of different field conditions, crop types, inoculant, and soil conditions. Multilocational testing, All-India Coordinated Trials, and other field trials in *C. cajan*, *P. vulgaris*, *A. hypogaea*, *G. max*, and *C. arietinum* have been reported to increase in grain yields by 14–30% due rhizobial inoculation (Khurana and Dudeja 1994; Annapurna and Balasundaram 1995). One ton *Rhizobium* biofertilizer is reported to provide 100 t N/ha, with the application dose of 0.5 kg per ha (Motsara et al. 1995).

14.2.1.2.1 Response of Pulses to *Rhizobium*

Inoculation response of pulses is far below the desirable level, mostly inconsistent and dependent on many variables. Estimates of N-fixation by different pulse crops with respect to India are presented in Table 14.2. Most cultivated soils to pulses are known to harbor *V. unguiculata* group of rhizobia, and nodulation surveys indicate a need for inoculation every season for the majority of the pulses grown in India. The competition of inefficient native strains to efficient inoculants strains appears to be a bottleneck in realizing higher yields from *Rhizobium* inoculation. The yield increase due to inoculation in *C. cajan* reported to be varied from 1.2% to 20.3%, 8 to 47.8%, and 1.8 to 26.4% in 1992, 1993, and 1994, respectively, in different locations in India. The grain yield increase also appears to be an interaction of varieties and strains of *Rhizobium* (Brahmaprakash and Hegde 2005; Dhakal et al. 2016).

Rhizobial numbers are the primary determinant of the number of nodules formed. Hence, it is essential to evaluate the native rhizobial population in soil in order to predict legume response to rhizobial inoculants. Depending upon the overall nodule formation in *C. arietinum*, a rating index has been prepared indicating poor (1–10 nodules/plant), moderate (11–20 nodules/plant), good (21–30 nodules/plant), and very good nodulation (>30 nodules/plant). The multilocational trial for *C. arietinum*

Table 14.2 Estimates of nitrogen fixation in different pulses

Crop	N-fixed (kg/ha per year)
Cicer arietinum	26–63
Lens culinaris	35–100
Cajanus cajan	68–200
Vigna radiate	50–55
Pisum sativum	46

Modified: Wani and Lee (1991) and Bhata and vsh (2016)

nodulation by the native/indigenous rhizobia in India reveals that 55.7% of the locations had poor nodulation, 29.8% of the locations moderate, 12% good, and only 2.5% of the locations showed very good nodulation. Low population index (LPI) of native rhizobia dictates the need for seed inoculation with rhizobial inoculants. Moreover, rhizobial population dynamics in the soil is considerably influenced by soil moisture. The locations near to a canal with the provision of sprinkler irrigation showed better nodulation in *C. arietinum* as compared to sand dunes of Loharu/Badra region of Haryana state (Khurana et al. 1997a, b).

A complementary, coordinated effort on the part of plant breeders and microbiologists is now necessary to successfully select a high-yielding variety with elevated N-fixing abilities for sustainable agriculture. A multidisciplinary coordinated research project on pulse crops such as *C. cajan*, *C. arietinum*, and MuLLaRP (mung (*V. radiata*), urd (*V. mungu*), lentil (*L. culinaris*), *Lathyrus* (*Lathyrus sativus*), rajma (*Phaseolus vulgaris*), and pea (*P. sativum*)) is in operation in India funded by Indian Council of Agricultural Research (ICAR), in many different centers for research on improvement of several aspects of these pulses. The emphasis in this project is on crop improvement, crop production, and plant protection of these legumes. The response of pulse crops to *Rhizobium* inoculation under different agroclimatic conditions is given in Table 14.3. Depending on agroclimatic conditions, the variety planted and pest control measures undertook increased yields from 0% to 75% over controls under All-India Coordinated Trials of pulses (Rewari 1984, 1985). Further, field experiments have conclusively demonstrated the addition of N to soil due to pulses cultivation.

Dudeja and Khurana (1999) demonstrated an increase in grain yield up to 40% with a simultaneous savings of 40 kg of N as urea on inoculating local cultivar of *C. arietinum* with mesorhizobia over uninoculated control through multilocational testing. Inoculation technology in *C. arietinum* (Wani et al. 1995; Bhattari et al. 1997), *Lens culinaris* (Bhattari et al. 1997), and *Cajanus cajan* (Khurana and Dudeja 1994) recorded varied yields across the world despite numerous trials that indicate enhanced growth and grain yield in response to inoculation of many pulse crops (Table 14.4) and reports from regions such as South Asia of poor nodulation in >40% in farmers' field. Average values for % Ndfa (N derived from the air) through legume-rhizobia symbiosis for the pulses are 63 in experiments and 65 in farmer's field which is the second highest to *Vicia faba* and *Lupinus* (Herridge et al. 2008). Dudeja and Khurana (2001) determined the availability of N to the extent of 80 kg of N/ha or above made by *Mesorhizobium* and particularly by biological N-fixation by nodulating cultivar ICC 435 of *Cicer arietinum* and its non-nodulating mutant 435 M. The grain yield in their multilocational trial recorded to the tune of 65–401 kg/ha, corresponding to 9–33% increase in grain yield over the uninoculated control. Overall, mean depicted that an average increase of 120–250 kg of grain yield could be achieved by applying an efficient inoculant strain costing less than US$0.40 under different conditions (Dudeja et al. 2011).

Globally legume inoculants can be divided between the relatively sophisticated markets of North and South America, Europe, and Australia which are dominated by a small number of manufacturers producing consistently high-quality inoculants

Table 14.3 Response of pulse crops to *Rhizobium* inoculation under different agroclimatic conditions

Crop	Location	Increase (%) in grain yield over uninoculated
Vigna mungo	Pudukkottai, Tamil Nadu	4.0–21.0
	Dholi, Bihar	11.0–29.0
	Pantnagar, Uttar Pradesh	17.0–21.0
	Varanasi, Uttar Pradesh	0.14–2.32
Vigna radiata	Lam, Andhra Pradesh	1.4–75.0
	Delhi	10.0–49.0
	Pantnagar, Uttar Pradesh	4.0–15.0
	Dantiwada, Gujarat	0–3.5
	Hisar, Haryana	<1.0
	Kovilpatti, Tamil Nadu	12.5
	Jodhpur, Rajasthan	<1.0
Cajanus cajan	Hisar, Haryana	5.0–25.0
	Pantnagar, Uttar Pradesh	2.0–25.0
	Sardarkrushinagar, Gujarat	9.0–21.0
	Sehore, Madhya Pradesh	13.0–29.0
	Rahuri, Maharashtra	20.0–41.0
	Hyderabad, Telangana	<1.0
	Kovilpatti, Tamil Nadu	40–47.2
Cicer arietinum	Varanasi, Uttar Pradesh	4.0–19.0
	Hisar, Haryana	24.0–43.0
	Dholi, Bihar	25.0–42.0
	Delhi	18.0–28.0
	Sehore, Madhya Pradesh	20.0–41.0
	Dahod, Gujarat	33.0–67.0
	Badnagar, Maharashtra	8.0–12.0
	Kovilpatti, Tamil Nadu	4.0–8.2
	Indore, Madhya Pradesh	4.0–8.2
	Rewa, Madhya Pradesh	2.9–22.0
	Varanasi, Uttar Pradesh	0–13.5
Lens culinaris	Pantnagar, Uttar Pradesh	4.0–26.0
	Ludhiana, Punjab	No response
Cyamopsis tetragonoloba	Agra, Uttar Pradesh	11.2–16.6
	Jhansi, Uttar Pradesh	3.7–8.6
	Jodhpur, Rajasthan	<1.0
Macrotyloma uniflorum	Bangalore, Karnataka	<1.0
	Hyderabad, Telangana	<1.0
Vigna unguiculata	Hyderabad, Telangana	<1.0
Dolichos sp.	Hyderabad, Telangana	<1.0
Vigna aconitifolia	Jodhpur, Rajasthan	<1.0
Glycine max	Kovilpatti, Tamil Nadu	0.0–5.0
	Indore, Madhya Pradesh	0.0–1.6
	Rewa, Madhya Pradesh	0.0–5.0

Modified from: Rewari (1984, 1985)

Table 14.4 Summary of inoculation responses for pulses

Species	Number of trials	Significant yield enhancement due to inoculation compared to farmer practice (% of trials)
Vigna radiata	78	67
Cicer arietinum	87	49
Vigna mungo	33	64
Cajanus cajan	35	63
Lens culinaris	50	86

Modified: Peoples et al. (2009)

and the less sophisticated and more fragmented production of inoculants in Asia and Africa of variable quality (Singleton et al. 1997; Herridge 2008).

In Asia and Africa, limited local production and distribution of inoculants (Sattar et al. 1997) and poor inoculant quality (Singleton et al. 1997) are the major constraints. There may also be a lack of knowledge about inoculants by farmers and extension personnel (Hoa et al. 2002) and an inability to convince farmers of the merits of inoculation because of inconsistencies in crop response or an inability to demonstrate visible differences between inoculated and uninoculated treatments (Joshi 1994; Khurana and Dudeja 1994). Some of these limitations could be addressed with increased effort in training and education and especially with improved inoculant quality, emphasis in R&D on rhizobial strain selection and methods of inoculants production and application, private-sector investment, and quality control (Herridge 2008). However, economic restrictions that bear on resource-poor farmers may remain an insurmountable problem. The use of promiscuously nodulating *Glycine max* lines that nodulate and fix N with naturalized rhizobia and, therefore, requires no inoculation is an approach being evaluated in Africa; it represents a potential solution to the poor adoption of inoculant technology (Sanginga 2003). There seems no good reason why similar strategies should not apply to other legume crops.

BNF benefits not only the legumes themselves but also any intercropped or succeeding crop, reducing or removing the need for N fertilization. In soils with low mineral N content, N-fixing microorganisms provide ammonium into the legume biomass, allowing faster growth than their plant competitors. In contrast, if N is abundant, N-fixing microorganisms tend to be competitively excluded by non-fixing species because the N-fixation process is bio-energetically costly (Houlton et al. 2008). It means that there is a range of physiological and ecological situations that tend to constrain BNF in legume systems, mainly by the N demand of the plant and by the C: N stoichiometry of the ecosystem (Peoples et al. 2009; Varma and Meena 2016).

The type and size of rhizobial populations in the soil are decided by particular legume species grown most recently (Thies et al. 1995). For example, invariably high populations of *Bradyrhizobium* sp. in Alfisols of Karnataka State, India, were recorded when a host pulse was part of recent cropping systems (Table 14.5). However, the population of essential rhizobia dropped significantly when a particular host legume has been excluded from recent cropping system, thus necessitating inoculation of appropriate strains of rhizobia to ensure satisfactory and effective nodulation (Hegde 1994).

Table 14.5 Effect of cropping system on *Bradyrhizobium* population in Alfisols at six sites in Karnataka State, India

Cropping system	Rhizobia per g soil
Cereal-legume intercrop	7000
Cereal-legume rotation	34,000
Sole legume	63,000
Sole non-legume	87

Modified: Hegde (1994)

Table 14.6 Inoculation effect of pulses on the yield of subsequent crops

	Yield of subsequent crop (Mg ha^{-1})		
Previous crop	Inoculated	Uninoculated	Crop
Cajanus cajan	2.46	2.08	*Triticum aestivum*
Cicer arietinum	2.76	2.52	*Oryza sativa*
Vigna mungo	2.16	2.08	*Triticum aestivum*
Lens culinaris	2.56	2.26	*Oryza sativa*

Modified: Subba Rao and Tilak (1977)

The existence and persistence of root-nodule bacteria are much regulated by the presence or absence of host plant. Stressful edaphic factors like pH, temperature, aridity, excess of heavy metals, low clay content, soil salinity, and fallowing are often detrimental to the survival of most rhizobia in the absence of appropriate host (Giller 2001; Slattery et al. 2001; Howieson and Ballard 2004). Nearly at some stage, all farmers' fields shall encounter one or more such stresses described above. Despite the principle of rhizobial ecology that useful populations of rhizobia remain in the soil when appropriate legume has been a component of recent cropping system, rhizobial numbers are expected to decline drastically in the stressful soil. For instance, populations of *Rhizobium leguminosarum* bv. *viciae* were frequently <100 per g soil (0–10 cm) in acidic soils within 1 year of the growth of a well-nodulated crop of *P. sativum* (Marshall et al. 1993).

Under field conditions plant responses to *Rhizobium* have been quite variable and depended on soil conditions, quality of inoculums, and effectiveness of native rhizobia. *Rhizobium*-legume symbiosis could meet more than 80% N needs of the legume crop (Singh 2005). Experimental evidence indicated that 10–15% increase in the yield of pulse crops consequent to *Rhizobium* inoculation (Brahmaprakash et al. 2007). Legume yields may not always be augmented by biofertilization. N concentration in plant parts and grains may increase, or the benefits may be regarding residual N which will benefit the succeeding crops (Table 14.6) by increasing yields as a result of enrichment of soil N due to N-fixation and its conserving effect of legumes at some instances. *V. radiata*, *V. unguiculata*, *V. mungo*, and *C. arietinum* have been reported to leave about 30–40 kg N/ha for the subsequent crop, and *C. cajan* were found to be an exhaustive crop (Dudeja et al. 2011; Dhakal et al. 2015).

14.2.2 Multiple Microorganisms for Enhancing Pulse Productivity

Enhanced crop productivity can be achieved by inoculating rhizobia with other plant growth-promoting organisms such as phosphate solubilizers, *Bacillus*, *Pseudomonas*, arbuscular mycorrhizal fungi (AMF), etc.

14.2.2.1 Phosphate-Solubilizing Microorganisms

P is one of the essential nutrients for the crops, commonly present as inorganic forms such as compounds of calcium (Ca), iron (Fe), aluminum (Al), etc. and as organic forms such as compounds of phytins, phospholipids, and nucleic acids in the soil (Richardson 2001). A large portion of the available form of P present in the super phosphate and other chemical fertilizers is converted to insoluble forms soon after application to soil. So, the availability of P to crop plants is always a limiting factor. The dual inoculation of *Rhizobium* and phosphobacteria is an important practice for N and P nutrients for pulse crops (Balamurugan and Gunasekaran 1996). P is vital for nodule formation, better N-fixation, root development, and yield of legumes (Rooge et al. 1998; Nkaa et al. 2014; Meena et al. 2017b).

Tropical soil features P deficiency naturally. Further, most of the P gets fixed, making it unavailable to plant. It is estimated that 75% of P of superphosphate applied gets fixed, and only 25% is available for plant growth in tropical soil. There are some bacteria and fungi which can solubilize unavailable form of P to available form by the virtue of secretion of certain organic acids (lactic acid, succinic acid, acetic acid, fumaric acid) and are labeled as phosphate-solubilizing microorganisms (PSM). These can grow on insoluble tricalcium and rock phosphate. The cheaper source of rock phosphate (RP) like Mussoorie rock phosphate, Udaipur rock phosphate, etc., available in our country, can be used along with the PSM. A considerable amount of foreign exchange can be saved by application of PSM, as the raw material for the manufacture of superphosphate is imported. Inoculation of crops can solubilize applied phosphate varying from 40 to 50 kg P_2O_5/ha and increase crop yield by 10–20%. The commercially important PSM in biofertilizer industry is *Bacillus megaterium* var. phospaticum. This biofertilizer is recommended for all crops. One ton PSM inoculant provides 24 t P_2O_5/ha, with the application dose 0.5 kg/ha (Motsara et al. 1995; Khan et al. 2009).

14.2.2.1.1 Performance of PSM on Co-inoculation with Rhizobia

Efficient and economic use of P-fertilizers could be achieved by using PSM in pulse crops. P-uptake and P content were improved by the application of BioPhos (PSM inoculants) in many leguminous plants. The advantage of PSM to uninoculation in respect of nodulation, P-uptake, pod yield, and net utilization of pulse production was recognized. Seed inoculation with PSM noted the beneficial effect on the yield of crops was the saving by the use of 75:25 ratio of MRP: SSP (Agasimani et al. 1994). Additionally, application of PSM can substitute 25% of phosphate fertilizers. The combination of rock phosphate with PSM in field application has increased the yield of pulses. P solubilization and its release from rock phosphate by PSM were affected in higher P-uptake and dry matter yield in pulses (Sharma et al. 2013).

BNF depends significantly on the available form of phosphorous. Hence, co-inoculation of nitrogen (N) fixers and PSMs benefits the plant better (by providing both N and phosphorus (P)) than solo inoculation with either group of organisms. The dual inoculation of phosphobacterium (*Bacillus megaterium* var. *phosphaticum*) and *Rhizobium* significantly increased the plant height, nodule number, and nodule weight of black gram under field condition (Surendra et al. 1993; Balachandran and Nagarajan 2002). PSB can improve the competitive ability and symbiotic effectiveness of inoculated *Rhizobium* sp. in *Lens culinaris* (Kumar and Chandra 2008) and leghemoglobin content of nodules of *Cicer arietinum* (Tagore et al. 2013) under field conditions. This is because the P is an essential nutrient in the form of ATP for the enzyme nitrogenase of *Rhizobium* and plays a key role in the energetics of plant metabolism. Production of siderophores is the prominent factor for the biocontrol effect as suggested by Siddiqui et al. (2007). Gupta et al. (1992) demonstrated the possibility of saving of 10 kg P chemical fertilizer by entirely replacing with rock phosphate and PSB biofertilizer in the presence of *Rhizobium* in the field. Multilocational trials were conducted with 20 and 40 kg P_2O_5/ha as SSP with and without mesorhizobial and PSM inoculation during 1994–1996. Twenty percent increase in yield in *C. arietinum* was recorded by the use of SSP with biofertilizer inoculation (Khrurana et al. 1999). When no P source was used, inoculation with PSM inoculants enhanced the *C. arietinum* grain yield by 9.4%. Dual application of rock phosphate with PSM inoculants bettered the *C. arietinum* grain yield up to 34.8%, compared to only 24% and 9% with P source alone and without P, respectively. PSM inoculants on combination with DAP enhanced the grain yield of *C. arietinum* by 46.1%, compared to 30.7% with a P source alone. Utilization of PSM inoculants resulted in saving of about 20 kg P_2O_5/ha and hence is a better alternate source of P nutrient for pulses.

14.2.2.2 Arbuscular Mycorrhizal Fungi

Mycorrhiza is a symbiotic association of a special group of fungi with the roots of plants and that benefits the translocation and uptake of phosphorous in plants. Arbuscular mycorrhizal fungi (AMF) popularly known as VAM is the fungi that colonize roots of several crop plants, important in agriculture, horticulture, and tropical forestry. They are formed by the group of fungi that are usually present in all soils from the phylum *Glomeromycota*, including ten genera: *Paraglomus*, *Glomus*, *Gigaspora*, *Geosiphon*, *Scutellospora*, *Diversispora*, *Sclerocystis*, *Acaulospora*, *Entrophospora*, and *Archaeospora* (Schuessler et al. 2001; Redecker and Raab 2006). The photobiont is formed by more than 90% of all vascular flowering plant families with around 170 described species (Smith et al. 1997; Brundrett 2009). These are obligate symbionts and necessitate living host for their multiplication and growth. AMF presence improves the absorption surface area of roots and promotes plant growth by water uptake and improved P nutrition usually by absorption and translocation of soil solution P by their high-affinity P-uptake mechanisms (Muchovej 2001; Aggarwal et al. 2011). For instance, the P-depletion zone around non-mycorrhizal roots extends to only 1–2 mm, nearly the length of a root hair, whereas extraradical hyphae of AMF extend 8 cm or more beyond the

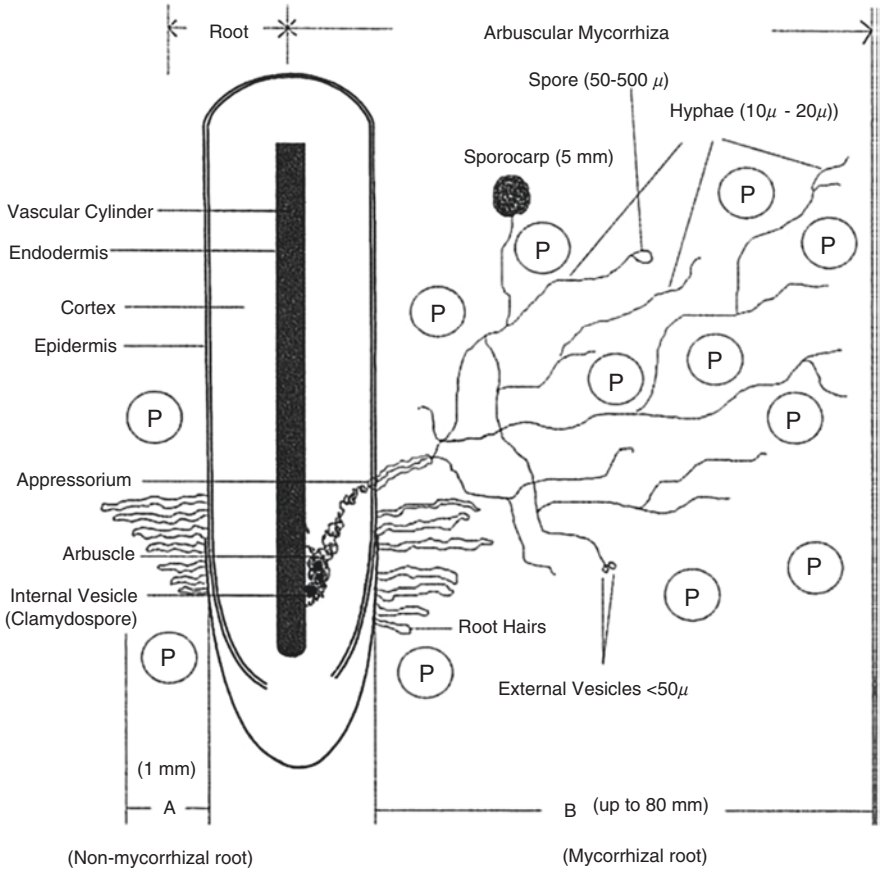

Fig. 14.4 Root colonized by AMF. Note the zone of P (or another nutrient) absorption by a non-mycorrhizal root (**a**) and by a mycorrhizal root (**b**) P-phosphate ion (Modified: Muchovej 2001)

root making the P in this greater volume of soil available to the host (Fig. 14.4). Additionally, increase in uptake of other nutrients, including N, S, B, Cu, K, Zn, Ca, Mg, Na, Fe, Mn, Al, and Si from the soil by AMF hyphae, has been documented (Clark and Zeto 2000). Indirectly BNF in legumes is expedited by AMF by facilitating the host plants with P and other immobile nutrients, such as copper (Cu) and zinc (Zn), essential for BNF (Li et al. 1991; Kothari et al. 1991; Clark and Zeto 2000). BNF can decline or even inhibited without AMF at low nutrient availability (Azcón et al. 1991; Muleta 2010). Table 14.7 enlists the direct or indirect effects of AMF on the growth and development of plants (Gosling et al. 2006; van der Heijden et al. 2008).

In the regime of low N and P availability, as encountered in several tropical soils, the possible transfer of nutrients from the mycorrhizal plant to another plant via AMF hyphal links developed inherently underground can take place (Heap and

Table 14.7 Effects of mycorrhizal fungi on crop productivity in organic farming systems

Direct effects	Indirect effects
Stimulation of plant productivity of various crops	Weed suppression
Enhanced seedling establishment	Stimulation of soil aggregation and soil structure
Nutrient acquisition (P, N, Cu, Fe, Zn)	Stimulation of N-fixation by legumes
Drought resistance	Suppression of some soil pathogens
Heavy metal/salt resistance	Stimulation of soil biological activity (phosphate solubilization)
	Increased soil carbon storage
	Reduction of nutrient leaching

Modified: Gosling et al. (2006) and van der Heijden et al. (2008)

Newman 1980). Research have established that AMF did increase N transfer from mycorrhizal legumes to another nonleguminous plant (Vankessel et al. 1985; Muleta 2010; Ram and Meena 2014).

14.2.2.2.1 Performance of AMF on Co-inoculation with Rhizobia

The occurrence of two symbionts in legume roots, *Rhizobium* bacteria and AM fungi which can sometime act synergistically, makes legumes a special case. This is well illustrated in *Stylosanthes guyanensis* grown in very low phosphate (2 ppm Olsen P) soil from the Brazilian cerrado (Mosse et al. 1976) where the combination of *Glomus fasciculatum* "E3" and rock phosphate greatly increased nodulation and N-fixation in addition to P-uptake and growth. Thus, the increased P-uptake by AMF stimulated the activity of *Rhizobium* which is well known to depend on an adequate supply of phosphate.

AMF are known to form a symbiotic relationship with a range of members of family Leguminosae (Pagano et al. 2007; Valsalakumar et al. 2007; Molla and Solaiman 2009). Studies reveal AMF colonizing *V. radiata* was of the trend *G. mosseae* (81%) followed by *G. microcarpum* (24%) and *G. margarita* (24%) and *Scutellospora* sp. (5%) (Valsalakumar et al. 2007). The range of distribution varied from a single species of AM fungus to three species belonging to two genera in one sample. In a study under five agroecological zones (AEZs) of Bangladesh, Molla and Solaiman (2009) surveyed the association of AMF in ten diverse leguminous crops and conclusively reported that *C. arietinum*, *V. radiata*, *L. culinaris*, and *V. mungo* were highly mycotrophic among all. In general, *Glomus* was most common, while *Sclerocystis* was the least prevalent genus in the study. The application of AMF inoculant in soils increased the growth and yields of legumes significantly under both greenhouse and field conditions. For instance, inoculation with AMF improved growth of *C. arietinum* and doubled P-uptake at low and intermediate levels of P in a pot experiment on sterilized low-P calcareous soil (Weber et al. 1992). Further, seed production in legumes significantly increased upon co-inoculation of mesorhizobia and AMF in multilocational field trials (Dudeja et al. 2011; Varma et al. 2017).

The majority of legumes have the ability to take part in a dual symbiotic interaction with rhizobia and AMF (Hazarika et al. 2000; Sprent 2001; Lodwig et al. 2003; Navazio et al. 2007). Rhizobia and AMF together play a key role in natural ecosystems and influence plant nutrition, productivity, resistance, and plant community structure (Cleveland et al. 1999; van der Heijden et al. 2006; Demir and Akkopru 2007). The tripartite symbiosis (between legumes-mycorrhiza and rhizobia) in a given ecosystem profoundly improved the overall performance of legumes (Subba Rao et al. 1986; Daniels-Hylton and Ahmad 1994; Barea et al. 2002; Xavier and Germida 2002; Zaidi et al. 2003; Stancheva et al. 2008). Generally, AMF improves phosphate nutrition of legume which, in turn, enhances plant growth and dinitrogen fixation. The effect of AM fungi on legumes often is duplicated by increasing P availability to non-AMF plants. Pot and field studies demonstrated that AMF inoculation enhanced BNF equivalent to that attainable by the application of phosphate fertilizer (75–100 kg P/ha). Recent studies have demonstrated that the two symbioses share some components of their developmental programs (Harrison 2005; Oldroyd et al. 2005; Navazio et al. 2007).

Positive effects of dual colonization of roots of legumes including pulses have been investigated by a number of workers. Most studies have concentrated on indirect relationships between AMF and rhizobia, in which a successful symbiosis has been measured as an increased uptake of nutrient elements, P in particular, by the plant (Ames and Bethlenfalvay 1987) and an increased dinitrogen fixation and nodule biomass (Fredeen and Terry 1988), but more direct non-nutritional effects of AM fungus-rhizobia interactions have also been reported (Bethlenfalvay et al. 1985).

Maximum nitrogenase activity and dry biomass of the legume resulted from co-inoculation of *Bradyrhizobium* sp. (*Vigna*) strain S-24 with *Scutellospora calospora* (Saxena et al. 1997). The nodulation competitiveness of the strain S-24 was significantly higher (60–63%) in the presence of *G. fasciculatum*, *G. mosseae*, and *S. calospora* when compared to nutrient with a single inoculation of S-24 (51%).

Jia et al. (2004) investigated the effects of the interactions between the microbial symbionts (*Rhizobium* and AMF) on N and P accumulation by broad bean (*Vicia faba*) and how increased N and P content influence biomass production, leaf area, and net photosynthetic rate. The AM fungus was found to promote biomass production and photosynthetic rates by increasing P/N accumulation, and an increase in P was consistently correlated with an increase in N accumulation and N productivity, expressed regarding biomass and leaf area. Photosynthetic N use efficiency, irrespective of the inorganic source of N (e.g., NO_3-), was enhanced by increased P supply due to AMF colonization. However, the presence of *Rhizobium* significantly declined AMF colonization irrespective of N supply; without *Rhizobium*, AMF colonization was higher in low-N treatments. The presence or absence of AMF did not have a significant effect on nodule mass, but high N with or without AMF led to a significant decline in nodule biomass. Furthermore, plants with the *Rhizobium* and AMF had higher photosynthetic rates per unit leaf area.

Geneva et al. (2006) reported that the dual inoculation of pea plants with *G. mosseae* or *G. intraradices* and *R. leguminosarum* bv. *viceae* strain D 293 significantly

increased the plant biomass, photosynthetic rate, nodulation, and N-fixing activity in comparison with a single inoculation of *R. leguminosarum* bv. *viceae* strain D 293. In addition, the co-inoculation significantly increased the total P content in plant tissues, acid phosphatase activity, and percentage of root colonization. Among all microbial pairing, the mixture of *R. leguminosarum* and *G. mosseae* was most effective at low P level, while *G. intraradices* inoculated with *R. leguminosarum* was most effective at higher P level, as also reported for *L. culinaris* (Xavier and Germida 2002). In other greenhouse trial, Mehdi et al. (2006) reported that the effects of AMF (*G. mosseae* and *G. intraradices*), rhizobial (*R. leguminosarum* bv. *viciae*) strains, and P- (superphosphate and phosphate rock) fertilizers considerably increased the dry biomass of shoots and seeds, P and N contents (shoots and seeds) of *L. culinaris* cv. "Ziba" plants, and percent of root colonized by AMF. The rhizobial strain possessing P-solubilizing ability showed a more beneficial effect on plant growth and nutrient uptake than the strain without this activity, although both strains had similar N-fixing efficiency. Moreover, the P-uptake efficiency was increased when P-fertilizers were applied along with AM fungi and/or P-solubilizing rhizobial strains emphasizing the remarkable importance of dual inoculation in the improvement of plant growth responses as also reported by Zarei et al. (2006) and Meena et al. (2013) for rhizobium-mycorrhizae inoculated *L. culinaris* plants.

In another study, Singh et al. (1991) and Boby et al. (2008) demonstrated the effect of live yeast cells (*Saccharomyces cerevisiae*) on nodulation and dry biomass of shoot and roots of legumes such as *Glycine max*, *L. leucocephala*, *C. cajan*, *Phaseolus aureus*, *Phaseolus mungo*, and *V. unguiculata* in the presence of both AMF and *Rhizobium* strains. The results indicate that inoculation with live yeast cells remarks ably enhanced the measured plant parameters. Root infection (native AMF) and the formation of vesicles, arbuscules, and spores were also increased with yeast inoculation. The increase in the parameters, however, varied with legumes and the type of yeast culture.

Legumes generally have less extensive root systems than graminaceous plants, for example, and many are poor foragers for soil phosphate. In addition, many strains of *Rhizobium* found in culture collection are adapted to media containing 100 times more P than is usual in the soil solution (Munns and Mosse 1980) and can require at least 0.1% P in the shoot tissues to produce nodules (Mosse et al. 1976). Some indigenous rhizobia, on the other hand, can nodulate at lower concentrations of P to which they are adapted (Mosse 1977), an important consideration in dual inoculation of pulses in P-deficient tropical soils.

14.2.2.3 Plant Growth-Promoting Rhizobacteria and Biocontrol Agents

Rhizosphere microorganisms closely associated with roots with beneficial properties are called plant growth-promoting rhizobacteria (PGPR). PGPR includes a diverse group of soil bacteria that can improve host plant growth by interacting with other soil organisms, thereby either by promoting the growth of beneficial microbes such as rhizobia or phosphate solubilizers or plants directly or by inhibiting the growth of pathogenic bacteria (Vejan et al. 2016). Solo inoculation of rhizobia improved the

yield by 12.4% compared to dual inoculation with PSB and PGPR by 22.1% increase in grain yield of *C. arietinum* in multilocational trials (Dudeja et al. 2011).

The effects of *G. intraradices*, *Rhizobium* sp., and *Pseudomonas striata* on the root-rot disease complex of *C. arietinum* caused by *Meloidogyne incognita* and *Macrophomina phaseolina* were observed. Dual inoculation of *G. intraradices* with *P. striata* plus *Rhizobium* to pathogen-inoculated plants caused a greater increase in plant biomass yield and a number of pods, chlorophyll, N, P, and potassium contents than by inoculation of *G. intraradices* plus *Rhizobium* or *G. intraradices* plus *P. striata* (Akhtar and Siddiqui 2008, 2009). Microbial inoculants (*Mesorhizobium*, PSB, and PGPR) with *Trichoderma viride* enhanced the productivity of *C. arietinum* (Dudeja et al. 2011; Yadav et al. 2017).

14.3 Formulations of Biofertilizers for Sustainable Agriculture

The success of inoculation technology depends on two factors such as the microbial strain and inoculants formulation. In practical terms, the formulation determines potential success of inoculants (Fages 1992). Inoculant (synonym for biofertilizer) is prepared containing beneficial microorganisms which enhance plant growth. As strains of same microbial species share many physiological properties, the technological progress thus developed for a particular strain is readily adaptable to another strain of some species with only minor modifications (Bashan 1998). Although formulation is vital in successful commercialization of biofertilizer, research in this area is meager. In addition to limited availability of published scientific information with regard to inoculant formulation, the information available is fragmented (Xavier et al. 2004). A survey of a bibliographic database of scientific literature shows that major emphasis was given to the development of improved strains through different approaches. Indeed many such strains have been constructed and granted patent in many developed countries but failed to appear on the commercial market, perhaps because of inappropriate formulation.

Development of improved formulations often rests with inoculant manufacturer's research and development facility which is primarily located in developed countries where target market exist, but they fail to consider the unique problems in applying these inoculants in developing countries. The most important characteristic common to most of biofertilizers is the unpredictability of their performance. To harness the benefits of biofertilizers in agriculture, the consistency of their performance must be improved (Wani and Lee 2002).

Bacteria introduced to soil may fail to establish in sufficient numbers in the rhizosphere because of competition from native numbers, and little is known about the factors controlling competitiveness of bacterial strains, more so under field conditions. Rhizosphere is the region of the soil under the influence of plant roots. Agricultural practices in developing countries and under semiarid conditions are two examples wherein biofertilizers may find their greatest challenges. Low-input agriculture is often followed by the farmers of developing countries in which

fertilizers, pesticides, and agro-technical machinery are least utilized. Application or uses of biofertilizers in such systems require additional infrastructure, cost, labor, and technical knowledge. Drought phenomenon often reduces the population of beneficial microorganisms in the soil. However, ample scope resides in the development of microbial inoculants for contributing immensely in a stress situation (Bashan 1998; Trivedi et al. 2012). Most important constraints for adoption of biofertilization in India have been attributed to poor quality of inoculants produced, lack of knowledge about inoculation technology for extension personnel and farmers, effective inoculants delivery/supply system, and lack of committed policy to exploit biofertilizers successfully (Wani and Lee 1991; Bodake et al. 2009; Jangid et al. 2012)

14.3.1 Inoculant Formulations

Formulation step is a vital aspect for producing microbial inoculants and determines the success of a biological agent. Formulation typically consists of establishing viable microorganisms in a suitable carrier together with additives that aid in stabilization and protection of microbial cell during storage, transport, and at the target. The formulation should also be easy to handle and apply so that it is delivered to target in most appropriate manner and form, one that protects microorganisms from harmful environmental factors and maintains or enhances the activity of the organisms in the field. Therefore, several critical factors including user preference have to be considered before delivery of a final product (Xavier et al. 2004; Meena and Meena 2017).

14.3.1.1 Carriers for Inoculant Formulations

A suitable carrier plays a major role in formulating microbial inoculants. Carriers are inert materials, used for transporting microbes from the laboratory to land. Carrier is a delivery vehicle which is used to transfer live microorganism from an agar slant of laboratory to a rhizosphere. A good quality inoculant should be made of a superior carrier material. Hence, Smith (1992) has listed the characters of a superior-quality carrier material for microbial inoculants, which includes:

- High water-holding and water-retention capacity
- No heat of wetting
- Nearly sterile, chemically, physically, and uniformly
- Nontoxic in nature, easily biodegradable, and not polluting
- Nearly neutral pH or easily adjustable
- Supports growth and survival of microorganisms
- Amenable to nutrient supplement
- Rapid release of microorganisms in soil
- Manageable in mixing, curing, and packaging operations
- Available in powder or granular form in adequate quantities and at reasonable cost

Considering the above characteristics, it is clear that not a single universal carrier is available which fulfill all the desirable features, but good ones should have as many as possible. Peat was the carrier of choice and most commonly used for *Rhizobium* inoculants worldwide for decades. Despite its popularity, variability in quality which is dependent on source, the release of antibacterial agents due to sterilization often poses a threat to biofertilizer production (Chao and Alexander 1984), and its availability is restricted to a very few countries. All these factors have forced researchers to look for alternative carrier materials. Some of the alternative carriers researched for biofertilizers which include lignite and coal (Kandasamy and Prasad 1971; Dube et al. 1980) clays and inorganic soils (Chao and Alexander 1984), compost, farm yard manure, soybean meal (Iswaran et al. 1972), wheat bran (Jackson et al. 1991), press mud (Philip and Jauhri 1984), spent agricultural waste material (Sadasivam et al. 1986), and spent mushroom compost (Bahl and Jauhri 1986). Apart from these, many other synthetic and inert materials like vermiculite (Paau et al. 1991; Sparrow and Ham 1983), perlite, ground rock phosphate, calcium sulfate, polyacrylamide gels (Dommergues et al. 1979), and alginate (Bashan 1986; Jung et al. 1982) have also been evaluated. Most of the evaluated carriers are either naturally abundant resources or available waste materials. Little research has have been conducted with objectives of synthesizing a carrier with superior characteristics (Bashan 1998). Biofertilizers are commonly designed for seed treatment and direct application to the soil. Hence, delivery of biofertilizers to crops is vital to determine the formulation, for instance, powder for seed treatment granulated for soil application (Walter and Paau 1993; Verma et al. 2015a, b).

Principal drawbacks of solid carrier-based inoculants originate from a great variability in quality of carriers which is source dependent, and composition of carriers is undefined and complex. The quality of carrier material to a great extent affects the final product and causes trouble in inoculant dosage, storage conditions (Van Elsas and Heijnen 1990), and difference of inoculant effectiveness between different manufacturers and batches from the same manufacturer (Bashan 1998). Carrier-based bacterial inoculants often confront lower tolerance for physical stress during storage, particularly for temperature differences. Some types of peat can even reduce plant growth (Huber et al. 1989). They are often prone to contamination that can reduce the shelf life of the inoculants (Van Elsas and Heijnen 1990; Fages 1992; Olsen et al. 1994a, b). Adhesion of inoculant to seeds is overcome by adhesives. However, incorporation of adhesive during application is labor and cost intensive (Smith 1995; Datta et al. 2017). Solid carrier-based inoculant production involves a significant amount of cost; labor; energy-intensive processing such as mining, drying, and milling; and neutralization before its use in a commercial production.

The carrier-based inoculants produced in India generally have a short storability, poor quality, high contamination, and erratic field performance. High-quality biofertilizers would be expected to have a higher population of desired microorganisms and sufficient viability and remain uncontaminated for longer period of storage. The carriers used in India are nearly inert material and form clumps upon drying, which leads to significant loss of viability. Seed is not a favorable environment for most of the plant growth-promoting bacteria, as they are soil bacteria, yet seed inoculation

is a common practice for microbial inoculation. The usual carrier-based biofertilizers do have some drawbacks for seed inoculation like seed coat damage, seed coat toxicity, the death of cells due to desiccation, and possible contact of microorganisms with agricultural chemicals. Today, advances in inoculant technology are concerned with improving quality, extending useful shelf life, and developing new formulations for use under less favorable conditions. Liquid inoculants and alginate-based granular formulations are two important new inoculant formulations which are an alternative to peat-/lignite-based ones.

14.3.1.2 Liquid Inoculants

Liquid inoculants are not the usual broth culture from a fermenter or water suspension of the carrier-based biofertilizers, as often made out to be. It is a special liquid formulation containing not only the desired microorganisms and their nutrients but also contains special cell protectant and amendments that promote cell survival in a package and after application to seed or soil. Various liquid media are being used to culture microorganisms. These media normally consist of carbon, N, and vitamin sources, which promote the growth of microorganisms. However, additives used in liquid inoculants improve quality of inoculants by increasing the population density and enhanced shelf life (Tittabutr et al. 2007).

Additives used in the preparation of liquid inoculants have been selected based on their ability to protect microbial cells in the package and on seeds extreme conditions such as high temperature, desiccation, and toxic condition of seeds and need chemicals. Most of the additives are high-molecular-weight polymers with good water solubility, non-toxicity, and complex chemical nature (Deaker et al. 2004; Ashoka et al. 2017) and can limit heat transfer and possess good rheological properties and high water activities (Mugnier and Jung 1985). Some of the polymers which are presently used in preparation of liquid inoculants include polyvinylpyrrolidone (PVP), methylcellulose, polyvinyl alcohol, polyethylene glycol, gum arabic, trehalose, glycerol, Fe-EDTA, sodium alginate, tapioca flour, etc. (Singleton et al. 2002; Tittabutr et al. 2007). Polyvinylpyrrolidone was known to bind toxic compounds present in seed exudates that are mobilized during inoculation and seed germination. PVP has a high water-binding capacity and appears to cause slow drying of an inoculant after application. PVP solution tends to coalesce into ridges on their seed coat as it dries, perhaps providing a thicker layer of protection than some other compounds. Its sticky consistency may also enhance cell and inoculants adherence seeds (Singleton et al. 2002). Sometimes seed-released compounds may bind iron in yeast extract, making it unavailable to cells. Supplementary iron may, therefore, replace Fe bound by seed exudates (Ali and Loynachan 1990). Glycerol has a high water-binding capacity and may protect cells from effects of desiccation by slowing the drying rate. Its flow characteristics appear to promote rapid and even coating on seeds (Al-Rashidi et al. 1982; Mary et al. 1985). Trehalose is widely reported to enhance cell tolerance to desiccation, osmotic, and temperature stress. It acts by stabilizing both enzymes and cell membranes, is a compatible osmoticum as well, and is readily manufactured by *Bradyrhizobium* given ideal conditions (Streeter 1985; Lippert and Galinski 1992). Addition of PVP in a medium was known to

protect both fast- and slow-growing *Rhizobium* (Bushby and Marshall 1977). Bushby and Marshall (1977) and Vincent et al. (1962) have shown that addition of maltose (9%) and montmorillonite clay could protect *Rhizobium* against high temperature and desiccation. Polymers that are soluble in liquid inoculant formulations are convenient for batch processing of inoculants and make seed application a simpler process for farmers. Liquid *Rhizobium* inoculants prepared with PVP as an osmoprotectant had improved shelf life, nodulation, and N-fixation on par with lignite-based inoculants in cowpea.

Liquid inoculants can be produced by a simple fermentation process, packed directly from the fermenter aseptically, and stored. It minimizes the production cost by avoiding processing and sterilization of solid carrier material. The complete sterilization could be achieved with liquid formulations, and any contamination during the storage can be easily detected. Liquid inoculants could be produced with minimum labor, space, and energy, and also the quantity of inoculum required is less compared to carrier-based formulations, hence easier for farmers to handle. The first yardstick to measure the quality of biofertilizer is the viable cell density of desired microorganisms which essentially provides adequate number of microorganisms on each seed. The liquid inoculants developed were known to have a population of *Rhizobium* sp. and PSB up to the level of 108 cells/ml (Sridhar et al. 2004; Vithal 2004; Dayamani 2010; Velineni and Brahmaprakash 2011; Kumar et al. 2016). A higher population of microorganisms are often prescribed in formulation to compete with native *Rhizobium* and to offset death of cells due to biotic and abiotic stresses. Since the liquid biofertilizer has high cell count, each seed receives more than thousands of cells. Additives in liquid biofertilizer protect the cells on the inoculated seeds against toxicity, desiccation, and osmotic shock (Vithal 2004). The storage and transportation conditions are not congenial many a times for the bioinoculants as temperature in many parts of the country may reach up to 45 °C; in such condition the quality of biofertilizer will decline drastically. Liquid biofertilizers were known to have more than 1 year shelf life compared to carriers. Studies have revealed that these liquid inoculants can be stored without losing viability in high-temperature (45 °C) conditions also (Vithal 2004). Imposition of stress to bacteria results in an adaptive response. This necessitates changes in metabolic processes in cells, which are then reflected in an alteration of protein profiles (Saxena et al. 1996). Synthesis of additional 19 salt-stress proteins (SSPs) in *Rhizobium* (40–52 kDa) and synthesis of 19 heat-shock proteins (ranging from 8 to 60 kDa) in *Bradyrhizobium japonicum* at 43 °C have been reported (Munchbach et al. 1999); *Bradyrhizobium* sp. (*Arachis*) grown at room temperature in liquid inoculant synthesized 60 and 47 kDa proteins of higher intensity but the same proteins of lower intensity in yeast extract mannitol broth (YEMB). *Bradyrhizobium* sp. (*Arachis*) on exposure to heat stress showed the presence of bands of same proteins (60 and 47 kDa) in liquid inoculant. Similarly, under salt stress (0.05 M NaCl), *Bradyrhizobium* sp. (*Arachis*) grown in liquid inoculant synthesized the extra proteins of 66 kDa but not in YEMB (Brahmaprakash et al. 2007). This kind of mechanisms provides potential to grow at different types of soil as we know that performance of inoculants depends largely on soil conditions. The amount of

inoculant needed for seed inoculation is less, and there is no need of any sticker material unlike carrier-based inoculants. Liquid inoculants can easily be adapted to advanced seeding equipments; since it can be sprayed onto seeds, it passes through seed drill and dries before it travels into the seed bin on the planter.

14.3.1.3 Field Response of Liquid *Rhizobium* Inoculants Formulation

Liquid rhizobial formulations are comparable to that of peat-based products performance under field conditions (Hynes et al. 2001). The field efficiency of the liquid inoculant was tested on farm trials under different agroclimatic conditions of India for two successive years in four different legumes like *C. cajan*, *A. hypogea*, *C. arietinum*, and *G. max*. Results of trials showed that liquid *Rhizobium* inoculants performed better than the carrier-based *Rhizobium* inoculants (Brahmaprakash et al. 2007; Meena et al. 2016). There is a need to improve formulation for better field performance. In this direction the research in progress indicates that solid carrier materials have been replaced with microbe-friendly liquid formulations. Further, a formulation containing a consortium is to be developed for field application.

14.3.1.4 Polymer Entrapped Inoculants Formulation

Polymers proved to be potential bacterial carriers in biofertilizers that offered substantial advantages over peat (Jung et al. 1982). These formulations encapsulate living cells, protect microorganisms against many environmental stresses, and release them to the soil, gradually but in large quantities, where the polymers are degraded by soil microorganisms. They can be dried stored at ambient temperatures for longer periods, can offer a consistent batch quality and a better defined environment for the bacteria, and can be manipulated easily according to the needs of specific bacteria. These inoculants can be amended with nutrients to improve short-term survival of bacteria upon inoculation, which is essential to the success of an inoculation process, especially with associative PGPB (Bashan 1998). However, a major limitation for the inoculation industry is that polymers are expensive compared to peat-based inoculants and require more handling by the industry (Fages 1992).

The encapsulation of microorganisms into a polymer matrix is still under research. At present there is no commercial bacterial product using this technology. The concept underlying immobilized microbial cells is to entrap beneficial microorganisms into a matrix. The formulation (bacterial matrix) is then fermented in a bacterial growth medium. Immobilized microbial cells are easy to produce, store, and handle during industrial operations. Encapsulated bacterial formulations in agriculture have two advantages (1) to temporarily protect the encapsulated microorganisms from the soil environment and microbial competition and (2) to release them gradually for the colonization of plant roots (Bashan 1986; Digat 1991; Bashan and Carrillo 1996).

14.3.1.5 Alginate-Based Formulations

Alginate is a commonly used polymer for encapsulation of microorganisms and is naturally occurring, composed of β-1,4-linked D-mannuronic acid and L-glucuronic

acid. It is available from different macroalgae (De Lucca et al. 1990) as well as several bacteria (Smidsrod and Skjak-Braek 1990). Alginate cost has recently dropped because of its massive production in the Far East, making it potentially more attractive to the inoculant industry. The preparation of beads containing bacteria is fairly easy and involves a multistep procedure (Fages 1992; Digat 1991).

The main advantages of alginate preparations are their nontoxic nature, biodegradability, and their slow release of microorganisms into soil (Fages 1992; Kitamikado et al. 1990). This technology was used to encapsulate the plant-beneficial bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* (Fages 1992), which were later successfully used to inoculate wheat plants under field conditions. The bacteria survived in the field long enough, and their populations were comparable to the survival of bacteria originating from other carrier-based inoculants (Bashan et al. 1987). Furthermore, the addition of clay and skim milk to the beads significantly increased bacterial survival over alginate beads alone. Alginate mixed with perlite was used to entrap *Rhizobium* (Hegde and Brahmaprakash 1992). Colonization of wheat roots by beneficial cells released from the beads was superior to that achieved by direct soil inoculation. These studies provide clear evidence that alginate beads are efficient slow-release carriers for plant inoculants, thus providing a protective environment in the soil. Several other alginate-based preparations have been tried for the encapsulation of AM fungi (Ganry et al. 1982), ectomycorrhizal fungi (Marx and Kenney 1982; Le Tacon et al. 1985), *Frankia* inoculation (Sougoufara et al. 1989), and fungi used as biocontrol agents against soil-borne pathogens (Fravel et al. 1985; Lewis and Papavizas 1985).

Alginate preparations may have solved many of the problems associated with traditional peat inoculant. These inoculant formulations may solve the problems associated with tropical, low-input agriculture. In many parts of the tropical region, there is always a chance of prolonged dryness after sowing and microbial inoculation. Alginate encapsulated formulations are already desiccated due to lower water activity; microorganisms will be allowed at metabolic activities and are released into soil only after sufficient moisture is available, which always coincide with the germination of seeds. Considering the cost involved in the production of alginate formulations, attempts have been made to amend these formulations with material like rock phosphate, cement, bentonite clays, granite powder, gypsum, lignite, and talc by which cost of production can be minimized besides adding bulkiness formulation (Lewis and Papavizas 1985; Meena et al. 2015b).

14.3.2 AMF Inoculants

The AMF being an obligate symbiont, there are many constraints in its large-scale commercial production and application. The only method of production in association with host plant by pot culture, as the production of AMF in the artificial media, has met with little or no success. There are different types of AMF inoculum required for different purposes. The spores of AM fungi are used as inocula generally for experiments in vitro conditions. Large-scale production of spores is difficult (Bagyaraj et al. 2002).

14.3.2.1 Infected Root Inoculums

Large-scale production of the infected root is possible in aeroponic culture. Infected roots contain internal mycelium and external mycelia (may have spores).Infected roots colonize a host after 1 or 2 days of inoculation. Root inocula without spores should be used within a week. In vitro reproduction of some fungi on cultured tissue roots has been demonstrated (Napamornbodi et al. 1988). The production process is difficult and expensive. Other problems are (a) infected root introduced as inocula which act as an attractive nutrient source for several saprophytic and parasitic microorganisms, (b) short survival time, and (c) large quantities of inocula required.

14.3.2.2 Soil-Based Inoculums

Soil inoculum is produced using traditional pot-culture techniques containing all AM fungal structures that are highly infective. The success of good soil inoculums production depends on the selection of host plant and the ambient conditions under which a defined AM fungus can be mass multiplied (Bagyaraj et al. 2002; Meena et al. 2013a). AMF can be mass multiplied through soil pot culture using *Chloris gayana* as host in conjunction with *Azospirillum*, with a view to reducing the length of production period (Bhowmik and Singh 2004; Bhowmik et al. 2015), and the root pieces along with the soil substrate is used as an inoculant.

14.3.2.3 Peat-Based Inoculants (Nutrient Film Technique)

AMF inocula obtained from pot cultures were incorporated into peat and compressed into blocks. Lettuce plants are allowed to grow in the peat block for 2–5 weeks, and then the blocks are transferred to nutrient film technique (NFT) channels (Cooper 1985). The NFT channels slope and nutrient solution flow at 200 ml per minute. Plants are allowed growing NFT system for 8–10 weeks. During this time, mass reproduction of the fungus takes place. The peat blocks are allowed to dry, chopped, and used as AMF inoculums. The shelf lives of such peat-based inoculants are around 6 months (Bagyaraj et al. 2002).

14.3.3 Mixed Bacterial Inoculants

Recent studies show a promising trend in the field of inoculation technology. A consortium of microorganisms that interact synergistically is presently being devised. Mixed inoculation of the plant with *Azospirillum* with other microorganisms enhanced the growth of plant compared to single inoculation (Bashan and Holguin 1997). For instance, plant growth can be increased by dual inoculation with *Azospirillum* and phosphate-solubilizing bacteria (Alagawadi and Gaur 1992; Belimov et al. 1995). *Azospirillum* is also considered to be a *Rhizobium*-"helper" stimulating nodulation, nodule activity, and plant metabolism, all of which stimulate many plant growth variables and plant resistance to unfavorable conditions (Andreeva et al. 1993; Itzigsohn et al. 1993). The synergistic interaction between *Rhizobium* and AMF in legume plants is well established (Bagyaraj 1984).

Co-inoculation with N-fixing bacteria and arbuscular mycorrhizal fungi creates synergistic interactions that may result in a significant increase in growth, P content, enhanced mycorrhizal infection, and an enhancement in the uptake of mineral nutrients such as N, P, K, Cu, Zn, and Fe (Bagyaraj 1984; Li and Huang 1987; Singh et al. 1990; Li et al. 1992; Garbaye 1994).

14.3.4 Formulations for Microbial Consortia: Inoculants for the Future

Despite progress in research on mixed inoculants, microbial inoculants with multiple organisms are not yet produced commercially. Until now, the research on mixed microbial inoculation was only confined to the development and inoculation of each bacterium in a separate formulation. But development of new inoculant formulation like polymer-entrapped desiccated inoculants has opened new vistas in mixed microbial inoculants. In this direction concept of "microbial consortium" assumes greater importance for sustainable agriculture. Microbial consortium is a group of microbial species that work together to carry out an overall reaction process, in our case beneficial organisms that together help promoting plant growth.

Feasibility of production of microbial consortium using *Rhizobium* and PSB using lignite, liquid, and alginate granules has been tested (Nethravathi and Brahmaprakash 2005; Shanker and Brahmaprakash 2004). It was observed that microbial consortium developed using alginate encapsulation was able to conserve the viability of both the organisms used for more than 6 months. But in liquid formulations, fast-growing bacilli had outnumbered the slow-growing rhizobia. There is a need to exercise caution in selecting the bacterial strains and formulation in the development of microbial consortium. Care should be taken to avoid bacterial strains which have antagonistic interactions among themselves. Alginate encapsulation a promising inoculant formulation for microbial consortia as they are desiccated formulation, microorganisms will be in the metabolically inactive state. The development of microbial consortium may minimize cost, labor, and energy involved in the production of inoculants. But more and more single strains microbial inoculants must be registered before inoculation industry can contemplate the development and commercialization of multi-bacterial inoculants (Polonenko 1994).

Indian agriculture, since the 1960s, has progressed tremendously due to the introduction of high-yielding varieties responding to high fertilizer inputs leading to enhanced food grains production. This high-input agriculture has also led to undesirable effects on environment and overall sustainability of the farming system such as adverse effects of agrochemicals. Fertilizer contamination of ground water has led to, over a period, eutrophication of lake and river water; caused decrease in oxygen content, death of aquatic life, and nitrate pollution; and increased emission of gaseous dinitrogen and metal toxicity. The nitrate toxicity caused health hazards such as birth defects, impaired nervous system, cancer, and methemoglobinemia (blue baby syndrome) (Knobeloch et al. 2000).

Table 14.8 Substitution of chemical fertilizers by biofertilizers

Sl. no.	Biofertilizers	Substitutes/ha per year	References
1.	*Rhizobium*	108.6–217.3 kg of urea	Mahdi et al. (2010)
2.	*Azolla*	20–40 kg urea/10 Mg	Mahdi et al. (2010)
3.	*Azospirillum*	60 kg urea in maize	Fulcheri and Frioni (1994)
4.	BGA	54–65 kg urea	Goyal and Venkataraman (1971) and Venkataraman (1981)
5.	*Frankia*	195 kg urea	Silvester (1975)

Modified from Brahmaprakash and Sahu (2012)
Calculated based on kg N-fixed × 2.17/ha per year

In this context, every unit of chemical fertilizers getting substituted by biofertilizers adds to sustainability and in the long run reduces the hazardous load of chemicals in the ecosystem. A rough estimate of the chemical fertilizers that may be substituted by biofertilizers is presented in Table 14.8. The future of inoculant technology and its benefits for sustainable agriculture depend on improving inoculant quality and effectiveness. Hence, the challenge is to develop and popularize inoculant formulation with long shelf life and effective in its response once inoculated, be it seed or soil. There is a need for extensive formulation like freeze-dried and fluid bed-dried inoculants. More recently mixed microbial inoculants have become popular; hence, further research work is required in this area, and also appropriate regulations and quality control guidelines are needed.

14.4 Future Perspectives of Biofertilizers in Pulse Production

Application of different biofertilizers as an essential factor of pulse cultivation is the new promising area nowadays. These inoculants are already being successfully utilized in India and few developing countries for pulse production and are expected to grow with time (Muleta 2010; Dudeja et al. 2011; Brahmaprakash and Sahu 2012). Therefore, it is logical to anticipate that in the future, the widespread utilization of biofertilizers will offer various potent strategies for overall development of agriculture. Table 14.9 summarily indicates selected examples of commercially available biofertilizers that have been used to improve the pulse production efficiency worldwide. However, more extensive utilization of biofertilizers will require addressing few issues with more attention and necessary actions to resolve the issues (Manoharachary 2004; Dudeja and Duhan 2005; Verma et al. 2015a).

1. Selection of most efficient *V. radiata* and *V. mungo* rhizobia among the predominant ones may prove to be better way of selecting efficient rhizobia with better competitive ability as has been observed in the case of *C. arietinum*.

Table 14.9 Commercially available biofertilizer worldwide for pulse production

Serial number	Type of biofertilizer with examples	References
1.	Nitrogen-fixing biofertilizers	Vessey (2003) and Dudeja et al. (2011)
	Rhizobium	
	Bradyrhizobium	
	Mesorhizobium	
2.	Phosphate-solubilizing biofertilizers	Khan et al. (2009)
	Bacillus	
	Pseudomonas	
	Aspergillus	
3.	Phosphate-mobilizing biofertilizer	Mehrvarz et al. (2008) and Jha et al. (2012)
	Arbuscular mycorrhizal fungi	
4.	Plant growth-promoting biofertilizers	Mehrvarz et al. (2008)
	Pseudomonas sp.	
5.	Phospho-bacterium and mycorrhizae	Suja (2008)
6.	*Rhizobium* and phosphobacterins	Dutta and Bandyopadhyay (2009)

Modified from Mahanty et al. (2016)

2. Comprehensive study on rhizobia infecting *V. radiata*, *V. Mungo*, *C*. Cajan, *Cyamopsis tetragonoloba*, and V. *unguiculata* using molecular techniques for proper classification and exploration of its multiple functions is needed. Currently PCR-based techniques deciphered the endophytic occurrence of these rhizobia in cereals and increase fertilizer use efficiency in these crops.
3. Identification of host genes involved in efficiency and competitiveness, as rhizobial genes involved in nodulation, dinitrogen fixation, and competitiveness are expressed in the host, so complementary genes in host should also be identified for further improvement in dinitrogen fixation. This may also include a robust pulse breeding program to improve the susceptibility of host to allochthonous symbiotic microbial species.
4. Elaborating the use of biofertilizers from laboratory and greenhouse experiments to large-scale commercial use will require a number of advanced new approaches for the growth, storage, shipping, formulation, and application of these bacteria and AM fungi. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation is highly deserved.
5. It is necessary to have requisite extension program to educate farmers and village level workers about the long-term benefit of biofertilizers. The ill effects of prolonged use of synthetic fertilizers should also be acknowledged to people side by side.
6. Invention of genetically modified organisms (GMOs) more efficacious in stimulating plant growth is required. Concurrently scientists will need to establish to both the public and regulatory agencies worldwide that GMOs do not present any new hazards or risk.

7. Establishing germination of AM fungi on agar media, multiplication and commercialization of efficient AM fungi; identification of marker genes and genetic mechanisms for effective "P" transport, for increased efficacy and adaptation to increase vigor yield, etc., establishing robust repository of AM fungi; and biotechnological application of AM fungi are the future lines proposed.
8. Investigation into the microbial persistence of biofertilizers in stressful soil environments is to be mandatory.
9. Assessment of biofertilizers on the basis of agronomic practices, soil, and socio-economic aspects under diverse agricultural production systems is desired for proper utility.
10. Quality control system for the production of biofertilizers and their use in the field guarantee and explore the benefits of plant-microorganism symbiosis. Hence, "Biofertilizer Act" and strict regulation for quality control in markets and application should be established.

14.5 Conclusion

Biofertilizers are low-cost inputs with high benefits in agriculture. There is a need to popularize this low-cost technology with the farming community to reap higher dividends. Although encouraging results have been obtained by pot and glasshouse experiments, the implementation of integrated nutrient management technologies by farmers is gloomy at field level. Incorporation of micronutrients is essential to increase pulse production in deficient soils of India. About 40% of the pulse-growing regions have low to the medium population of native *Rhizobium*. In these circumstances, productivity of pulse may be increased by 10–12% via seed inoculation with low-cost rhizobial biofertilizer. Biofertilizers supplementing P nutrition in agriculture may be vital in saving the much needed foreign exchange if we succeed in making the "fixed" P available to crops. AMF inoculant is promising to improve the supply of phosphate and micronutrients like zinc for a variety of pulse crops, while phosphate solubilizers are the best option in rainfed areas of poor P availability. Shortage of adequate quantity of quality biofertilizer is one of the chief limitations in the popularization of biofertilizers. Therefore, efforts are needed to ensure availability of critical inputs like quality biofertilizers, etc. at the state level. A combined effort between soil chemists, microbiologists, and agronomists is required to facilitate judicious use of inorganic and microbiological inputs to realize better yields while ensuring the agriculture remains sustainable.

References

Adesemoye AO, Kloepper JW (2009) Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85:1–12

Agasimani CA, Mudalgiriyapla, Sreenivasa MN (1994) Response of groundnut to phosphate solubilising microorganisms. Groundnut News 6:5

Aggarwal A, Kadian N, Tanwar A, Yadav A, Gupta KK (2011) Role of arbuscular mycorrhizal fungi (AMF) in global sustainable development. J Appl Nat Sci 3:340–351

Akhtar MS, Siddiqui ZA (2008) Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp. and *Pseudomonas striata*. Crop Prot 27:410–417

Akhtar MS, Siddiqui ZA (2009) Effects of phosphate solubilizing microorganisms and Rhizobium sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. Afr J Biotechnol 8:3479–3488

Alagawadi AR, Gaur AC (1992) Inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria on yield of sorghum [*Sorghum bicolor* (L.) Moench] in dry land. Trop Agric 69:347–350

Ali M, Gupta S (2012) Carrying capacity of Indian agriculture: pulse crops. Curr Sci 102:874–881

Ali FS, Loynachan TE (1990) Inhibition of *Bradyrhizobium japonicum* by diffusates from soybean seed. Soil Biol Biochem 22:973–976

Al-Rashidi RK, Loynachan TE, Frederick LR (1982) Desiccation tolerance of four strains of *Bradyrhizobium japonicum*. Soil Biol Biochem 14:489–493

Ames RN, Bethlenfalvay GJ (1987) Localized increase in nodule activity but no competitive interaction of cowpea rhizobia due to pre-establishment of vesicular arbuscular mycorrhiza. New Phytol 106:207–215

Andreeva IN, Red'kina TV, Ismailov SF (1993) The involvement of indole acetic acid in the stimulation of *Rhizobium*-legume symbiosis by *Azospirillum brasilense*. Russ J Plant Physiol 40:901–906

Annapurna K, Balasundaram VR (1995) Microbiological report on All India Coordinated Soybean Improvement Programme. ICAR, New Delhi

Ashoka P, Meena RS, Kumar S, Yadav GS, Layek J (2017) Green nanotechnology is a key for eco-friendly agriculture. J Clean Prod 142:4440–4441

Azcón R, Rubio R, Barea JM (1991) Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N2-fixation (15N) and nutrition of *Medicago sativa* L. New Phytol 117:399–404

Bagyaraj DJ (1984) Biological interaction with VA mycorrhizal fungi. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC Press, Boca Raton, pp 131–153

Bagyaraj DJ, Mehrotra VS, Suresh CK (2002) Vesicular arbuscular mycorrhizal biofertilizer for tropical forest plants. In: Kannaiyan S (ed) Biotechnology of biofertilizers. Narosa Publishing House, New Delhi

Bahl N, Jauhri S (1986) Spent mushroom compost as a carrier for bacterial inoculant production. In: Proceedings of the international symposium on scientific and technological aspects of cultivating edible fungi. The Pennsylvania State University, University Park

Balachandran D, Nagarajan P (2002) Dual inoculation of *Rhizobium* and phosphobacteria with phosphorus on black gram cv. Vamban 1. Madras Agric J 89:691–693

Balamurugan S, Gunasekaran S (1996) Effect of combined inoculation of *Rhizobium* sp., and phosphobacteria at different levels of phosphorus in groundnut. Madras Agric J 83:503–505

Barea JM, Azcon R, Azcon-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Anton Leeuw 81:343–351

Bashan Y (1986) Alginate beads as synthetic inoculants carriers for the slow release of bacteria that affect plant growth. Appl Environ Microbiol 51:1089–1098

Bashan Y (1998) Inoculants for plant growth promoting bacteria for use in agriculture. Adv Biotechnol 16:729–770

Bashan Y, Carrillo A (1996) Bacterial inoculants for sustainable agriculture. In: Pérez-Moreno J, Ferrera-Cerrato R (eds) New horizons in agriculture: agroecology and sustainable development. Proceedings of the 2nd international symposium on agroecology, sustainable agriculture and education. Colegio de Postgraduados en ciencias agricolas, Montecillo

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Bashan Y, Levanony H, Ziv-Vecht O (1987) The fate of field-inoculated *Azospirillum brasilense* Cd in wheat rhizosphere during the growing season. Can J Microbiol 33:107

Belimov AA, Kojemiakov AP, Chuvarliyeva CV (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil 173:29–37

Berg G, Zachow C, Müller H, Phillips J, Tilcher R (2013) Next-generation bio products sowing the seeds of success for sustainable agriculture. Agronomy 3:648–656

Beringer JE, Berwin AVB, Johnston Schulman JB, Hopwood DA (1979) The *Rhizobium*-legume symbiosis, in the cell as a habitat. University Press, Cambridge

Bethlenfalvay GJ, Brown MS, Stafford AE (1985) The glycine-glomus-rhizobium symbiosis: antagonistic effects between mycorrhizal colonization and nodulation. Plant Physiol 79:1054–1059

Bhardwaj D, Ansari MW, Sahoo RK (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13:66. http://www.microbialcellfactories.com/content/13/1/66

Bhata S, Umesh KB (2016) Estimating positive externalities of nitrogen fixation by pulses. Agric Econ Res Rev 29(2):201–209. https://doi.org/10.5958/0974-0279.2016.00048.3

Bhattari S, Maskey SL, Kama RL (1997) On-farm experiments on rhizobial inoculants in Nepal. In: Rupela OP, Johansen E, Herridge DF (eds) Extending nitrogen fixation research to farmers' fields. ICRISAT, Hyderabad

Bhowmik SN, Singh CS (2004) Mass multiplication of AM inoculum: effect of plant growth promoting rhizobacteria and yeast in rapid culturing of *Glomus mosseae*. Curr Sci 86:705–709

Bhowmik SN, Yadav GS, Datta M (2015) Rapid mass multiplication of *Glomus mosseae* inoculum as influenced by some biotic and abiotic factors. Bangladesh J Bot 44:209–214

Boby VU, Balakrishna AN, Bagyaraj DJ (2008) Interaction between *Glomus mosseae* and soil yeasts on growth and nutrition of cowpea. Microbiol Res 163:693–700

Bodake HD, Gaikwad SP, Shirke VS (2009) Study of constraints faced by the farmers in adoption of bio-fertilizer. Int J Agric Sci 5:292–294

Brahmaprakash GP, Hegde SV (2005) Nitrogen fixing in pigeonpea. In: Ali M, Shivkumar (eds) Advances in pigeonpea research. Indian Institute of Pulses Research, Kanpur

Brahmaprakash GP, Sahu PK (2012) Biofertilizers for sustainability. J Indian Inst Sci 92:37–62

Brahmaprakash GP, Girisha HC, Vithal N, Laxmipathy R, Hegde SV (2007) Liquid *Rhizobium* inoculants formulations to enhance biological nitrogen fixation in food legumes. J Food Legum 20:75–79

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77

Bulgarelli D, Schlaeppi K, Spaepen S, Loren V, van Themaat E, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838

Buragohain S, Sharma B, Nath JD, Gogaoi N, Meena RS, Lal R (2017) Impact of ten years of biofertilizer use on soil quality and rice yield on an inceptisol in Assam, India. Soil Res. https://doi.org/10.1071/SR17001

Burris RH, Roberts GP (1993) Biological nitrogen fixation. Annu Rev Nutr 13:317–335

Bushby HVA, Marshall KC (1977) Water status of rhizobia in relation to their susceptibility to desiccation and to their protection by montmorillonite. J Gen Microbiol 99:19–28

Chao WL, Alexander M (1984) Mineral soils as carriers for *Rhizobium* inoculants. Appl Environ Microbiol 47:94–97

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. Glob Biogeochem Cycles 13:623–645

Cooper AJ (1985) Crop production recirculating nutrient solution. Sci Hortic 3:35–38

Daniels-Hylton KDM, Ahmad MH (1994) Inoculation response in kidney beans (*Phaseolusvulgaris* L.) to vesicular-arbuscular mycorrhizal fungi and rhizobia in non-sterilized soil. Biol Fertil Soils 18:95–98

Datta R, Baraniya D, Wang YF, Kelkar A, Moulick A, Meena RS, Yadav GS, Ceccherini MT, Formanek P (2017) Multi-function role as nutrient and scavenger of free radical in soil. Sustain MDPI 9(8):402. https://doi.org/10.3390/su9081402

Dayamani KJ (2010) Formulation and determination of effectiveness of liquid inoculants of plant growth promoting rhizobacteria. PhD thesis, University of Agricultural Sciences, Bangalore, India

De Lucca AJ, Connick WJ Jr, Fravel DR, Lewis JA, Bland JM (1990) The use of bacterial alginates to prepare biocontrol formulations. J Ind Microbiol 6:129–134

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology- a review. Soil Biol Biochem 36:75–88

Demir S, Akkopru A (2007) Using of arbuscular mycorrhizal fungi (AMF) for biocontrol of soil borne fungal plant pathogens. In: Chincholkar SB, Mukerji KG (eds) Biological control of plant diseases. Haworth, Philadelphia, pp 17–37

Dhakal Y, Meena RS, De N, Verma SK, Singh A (2015) Growth, yield and nutrient content of mungbean (*Vigna radiata* L.) in response to INM in eastern Uttar Pradesh, India. Bangladesh J Bot 44(3):479–482

Dhakal Y, Meena RS, Kumar S (2016) Effect of INM on nodulation, yield, quality and available nutrient status in soil after harvest of green gram. Legum Res 39(4):590–594

Digat B (1991) A new encapsulation technology for bacterial inoculants and seed bacterization. In: Keel C, Koller B, Défago G (eds) Plant growth-promoting rhizobacteria – progress and prospects. IOBC/WPRS Bulletin, Zurich

Dixon ROD, Wheeler CT (1986) Nitrogen fixation in plants. Chapman and Hall, New York

Dommergues YR, Diem HG, Divies C (1979) Polyacrylamide entrapped *Rhizobium* as an inoculant for legumes. Appl Environ Microbiol 37:779–981

Dube JN, Mahere DP, Bawat AF (1980) Development of coal as a carrier for rhizobial inoculants. Sci Cult 46:304

Dudeja SS, Duhan JS (2005) Biological nitrogen fixation research in pulses with special reference to mungbean and urdbean. Indian J Pulses Res 18:107–118

Dudeja SS, Khurana AL (1999) Long term multiplication field evaluation of chickpea rhizobia in India. In: Proceedings of international symposium on long term fertilization trials as a basis for sustainable land use and quantification of matter cycles. UFZ-Bericht, Halle

Dudeja SS, Khurana AL (2001) Integrated management of N and P availability in chickpea through the use of Rhizobium and phosphate solubilizers. In: Proceedings of XIV international plant nutrition colloquium. Hannover

Dudeja SS, Narula N (2008) Molecular diversity of root nodule forming bacteria. In: Khachatourians GG, Arora DK, Rajendran TP, Srivastava AK (eds) Agriculturally important microorganisms, vol 2. Academic World International, Bhopal

Dudeja SS, Singh NP, Sharma P, Gupta SC, Chandra R, Dhar B, Bansal RK, Brahmaprakash GP, Potdukhe SR, Gundappagol RC, Gaikawad BG, Nagaraj KS (2011) Biofertilizer technology and pulse production. In: Singh A et al (eds) Bioaugmentation, biostimulation and biocontrol, soil biology. Springer, Berlin

Dutta D, Bandyopadhyay P (2009) Performance of chickpea (*Cicer arietinum* L.) to application of phosphorus and biofertilizer in laterite soil. Arch Agron Soil Sci 55:147–155

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

Fages J (1992) An industrial view of *Azospirillum* inoculants: formulation and application technology. Symbiosis 13:15–26

FAOSTAT (2009) Online interactive database on agriculture. FAOSTAT. www.fao.org

Fravel DR, Marois JJ, Lumsden RD, Connick WJ (1985) Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathology 75:774–777

Fredeen AL, Terry N (1988) Influence of vesicular–arbuscular mycorrhizal infection and soil phosphorous level on growth and carbon metabolism of soybean. Can J Bot 66:2311–2316

Fulcheri E, Frioni D (1994) *Azospirillum* inoculation of maize: effects on yield on a field experiment in Central Argentina. Biophysics 244:686–691

Galloway J, Raghuram N, Abrol YP (2008) A perspective on reactive nitrogen in a global, Asian and Indian context. Curr Sci 94:1375–1381

Ganry F, Diem HG, Dommergues YR (1982) Effect of inoculation with *Glomus mosseae on* nitrogen fixation by field grown soybeans. Plant Soil 68:321–329

Garbaye L (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210

Geneva M, Zehirov G, Djonova E, Kaloyanova N, Georgiev G, Stancheva I (2006) The effect of inoculation of pea plants with mycorrhizal fungi and *Rhizobium* on nitrogen and phosphorus assimilation. Plant Soil Environ 52:435–440

Giller KE (2001) Nitrogen fixation in tropical cropping systems. CAB International, Wallingford

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Goyal SK, Venkataraman GS (1971) Effects of algalization on high yielding rice varieties. II. Response of soil types. Phykos 10:32–38

Gupta SB, Vyas MK, Patil SK (1992) Effect of phosphorus solubilizing bacteria and thiram at different levels of phosphorus on soybean and soil micro flora. J Indian Soc Soil Sci 40:854–856

Harrison MG (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

Hazarika DK, Das KK, Dubey LN, Phookan AK (2000) Effect of vesicular arbuscular mycorrhizal (VAM) fungi and *Rhizobium* on growth and yield of green gram (*Vigna radiata* (L.)Wilczek.). J Mycol Plant Pathol 30:424–426

Heap AJ, Newman EL (1980) Links between roots by hyphae of vesicular arbuscular mycorrhizas. New Phytol 85:169–171

Hegde SV (1994) Population of cowpea rhizobia in farmers' fields in southern Karnataka: influence of cropping system, locations, and N-level. In: Rupela OP, Kumar Rao JVDK, Wani SP, Johansen C (eds) Linking biological nitrogen fixation research in Asia. ICRISAT, Hyderabad

Hegde SV, Brahmaprakash GP (1992) A dry granular inoculants of *Rhizobium* for soil application. Plant Soil 144:309–311

Herridge DF (2008) Inoculation technology for legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Nitrogen fixation: origins, applications and researchprogress. Nitrogen-fixing leguminous symbioses, vol 7. Springer, The Netherlands

Herridge DF, Peoples MB, Boddey RM (2008) Marschner review: global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Hoa NTL, Thao TY, Lieu P, Herridge DF (2002) N2 fixation of groundnut in the eastern region of south Vietnam. In: Herridge D (ed) Inoculants and nitrogen fixation of legumes in Vietnam. ACIAR proceedings. ACIAR, Canberra

Houlton BZ, Wang YY, Vitousek PM, Eield CB (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–330

Howieson J, Ballard R (2004) Optimising the legume symbiosis in stressful and competitive environments within southern Australia – some contemporary thoughts. Soil Biol Biochem 36:1261–1273

Huber DM, El-Nasshar L, Moore HW, Mathre DE, Wagner JE (1989) Interaction between a peat carrier and bacterial seed treatments evaluated for biological control of the take – all diseases of wheat (*Triticum aestivum* L.). Biol Fertil Soils 8:166–171

Hynes RK, Jans DC, Bremer E, Lupwayi NZ, Rice WA, Clayton GW, Collins MM (2001) *Rhizobium* sp. population dynamics in the pea rhizosphere of rhizobial inoculants strain applied in different formulations. Can J Microbiol 47:595–600

Iswaran V, Sen A, Apte R (1972) Plant compost as a substitute for peat for legume inoculants. Curr Sci 41:299

Itzigsohn R, Kapulnik Y, Okon Y, Dovrat A (1993) Physiological and morphological aspects of interactions between *Rhizobium meliloti* and alfalfa (*Medicago saliva*) in association with *Azospirillum brasilense*. Can J Microbiol 39:610–615

Jackson AM, Whipps JM, Lynch JM (1991) Production, delivery systems and survival in soil of four fungi with disease biocontrol potential. Enzym Microb Technol 13:636–642

Jangid MK, Khan IM, Singh S (2012) Constraints faced by the organic and conventional farmers in adoption of organic farming practices. Indian Res J Ext Educ Spec Issue 2:28–32

Jarvis BDW, van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet-Marel JC, Gillis M (1997) Transfer of *Rhizobium loti, Rhizobium huakuii, Rhizobium ciceri, Rhizobium mediterraneum* and Rhizobium tianshanense to Mesorhizobium gen. nov. Int J Syst Bacteriol 47:895–898

Jha MN, Kumar P, Chourasia SK (2012) Hope, hype and reality of biofertilizer. Fertil Technol 121:448–480

Jia Y, Gray VM, Straker CJ (2004) The influence of *Rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. Ann Bot 94:251–258

Joshi PK (1994) Field response of groundnut to *Bradyrhizobium* inoculation. In: Rupela OP, Kumar Rao JVDK, Wani SP, Johansen C (eds) Linking biological nitrogen fixation research in Asia. ICRISAT, Hyderabad

Jung G, Mugnier J, Diem HG, Dommergues YR (1982) Polymer-entrapped *Rhizobium* as an inoculant for legumes. Plant Soil 65:219–231

Kandasamy R, Prasad NN (1971) Lignite as a carrier of rhizobia. Curr Sci 40:496

Khan MZ, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of meta contaminated soils. Environ Chem Lett 7:1–19

Khrurana AL, Dudeja SS, Singh M (1999) Increasing the efficiency of phosphatic fertilizer with phosphate solubilizing bacteria to improve soil fertility and chickpea (*Cicer arietinum*) productivity. In: Proceedings of international symposium on long term fertilization trials as a basis for sustainable land use and quantification of matter cycles. UFZ-Bericht, Halle

Khurana AL, Dudeja SS (1994) On-farm experience in the use of rhizobial inoculants on pigeonpea in India. In: Rupela OP, Kumar Roo JVDK, Wani SP, Johansen C (eds) Linking biological nitrogen fixation research in Asia. ICRISAT, Hyderabad

Khurana AL, Namdeo SL, Patel BI, Dudeja SS (1997a) On-farm experiments on rhizobial inoculants – problems and possible solutions. In: Rupela OP, Johansen C, Herridge DF (eds) Extending nitrogen fixation researchto farmers' fields. ICRISAT, Hyderabad

Khurana AL, Namdeo SL, Patel BJ, Dudeja SS (1997b) On-farm experiments on rhizobial inoculants: problems and possible solutions. In: Rupela OP, Johansen C, Herridge DF (eds) Extending nitrogen fixation research to farmers' fields. Proceeding of managing legumes nitrogen fixation in cropping systems of Asia, ICRISAT Asia Center

Kitamikado M, Yamaguchi K, Tseng CH, Okabe B (1990) Methods designed to detect alginate-degrading bacteria. Appl Environ Microbiol 56:2939–2940

Kloepper JW, Lifshitz R, Schroth MN (1988) Pseudomonas inoculants to benefit plant production. In: ISI atlas of science. Institute for Scientific Information, Philadelphia

Knobeloch L, Salna B, Hogan A, Postle J, Anderson H (2000) Blue babies and nitrate-contaminated well water. Environ Health Perspect 108:675–678

Kothari SK, Marschner H, Romheld V (1991) Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil 131:177–185

Kumar R, Chandra R (2008) Influence of PGPR and PSB on *Rhizobium leguminosarum* Bv. viciae strain competition and symbiotic performance in lentil. World J Agric Sci 4:297–301

Kumar S, Sheoran S, Kumar SK, Kumar P, Meena RS (2016) Drought: a challenge for Indian farmers in context to climate change and variability. Progress Res Int J 11:6243–6246

Le Tacon F, Jung G, Mugnier J, Michelot P, Mauperin C (1985) Efficiency in a forest nursery of an ectomycorrhizal fungus inoculum produced in a fermentor and entrapped in polymeric gels. Can J Bot 63:1664–1668

Lewis JA, Papavizas GC (1985) Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of the fungi in the soil. Plant Pathol 34:571–577

Li CY, Huang LL (1987) Nitrogen fixing (acetylene reducing) bacteria associated with ectomycorrhizas of Douglas –fir. Plant Soil 98:425–428

Li XL, Marschner H, George E (1991) Acquisition of phosphorus and copper by VA–mycorrhizal hyphae and root-to-shoot transport in white clover. Plant Soil 136:49–57
Li CY, Massicote HB, Moore LVH (1992) Nitrogen-fixing *Bacillus* sp. associated with Douglas -fir tuberculate ectomycorrhizae. Plant Soil 140:35–40
Lippert K, Galinski EA (1992) Enzyme stabilization by ectoine type compatible solutes: protection against heating, freezing and drying. Appl Microbiol Biotech 37:61–65
Lodwig EM, Hosie AHF, Bourdés A, Findlay K, Allaway D, Karunakaran R, Downie JA, Poole PS (2003) Amino-acid cycling drives nitrogen fixation in the legume–Rhizobium symbiosis. Nature 422:722–726
Madigan MT, Martinko JM, Dunlap PV, Clark DP (2009) Brock biology of microorganisms, 12th edn. Pearson Benjamin, Cummings
Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, Tribedi P (2016) Biofertilizers: a potential approach for sustainable agriculture development. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-016-8104-0
Mahdi SS, Hassan GI, Samoon SA, Rather HA, Dar Showkat A, Zehra B (2010) Biofertilizers in organic agriculture. J Phytol 2:42–54
Manoharachary C (2004) Biodiversity, taxonomy, ecology, conservation and biotechnology of arbuscular mycorrhizal fungi. Indian Phytopathol 57:01–06
Marshall DI, Bateman JD, Brockwell J (1993) Validation of a serial-dilution, plant-infection test for enumerating *Rhizobium leguminosarum* bv. *viciae* and its application for counting rhizobia in acid soils. Soil Biol Biochem 25:261–268
Marx DH, Kenney DS (1982) Production of ectomycorrhizal fungus inoculum. In: Schenck NC (ed) Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul
Mary P, Ochin D, Tailliez R (1985) Rates of drying and survival of *Rhizobium meliloti* during storage at different relative humidities. Appl Environ Microbiol 50:207–211
Meena H, Meena RS (2017) Assessment of sowing environments and bio-regulators as adaptation choice for clusterbean productivity in response to current climatic scenario. Bangladesh J Bot 46(1):241–244
Meena KK, Meena RS, Kumawat MS (2013) Effect of sulphur and iron fertilization on yield attribute, yield, nutrient uptake of mungbean (*Vigna radiata* L.). Indian J Agric Sci 83(4):108–112
Meena VS, Maurya BR, Verma R, Meena RS, Jatav GK, Meena SK, Meena R, Meena SK (2013a) Soil microbial population and selected enzyme activities as influenced by concentrate manure and inorganic fertilizer in alluvium soil of Varanasi. Bioscience 8(3):931–935
Meena RS, Yadav RS, Meena H, Kumar S, Meena YK, Singh A (2015a) Towards the current need to enhance legume productivity and soil sustainability worldwide: a book review. J Clean Prod 104:513–515
Meena RS, Meena VS, Meena SK, Verma JP (2015b) Towards the plant stress mitigate the agricultural productivity: a book review. J Clean Prod 102:552–553
Meena H, Meena RS, Singh B, Kumar S (2016) Response of bio-regulators to morphology and yield of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] under different sowing environments. J App Nat Sci 8(2):715–718
Meena RS, Kumar V, Yadav GS, Mitran T (2017a) Response and interaction of *Bradyrhizobium japonicum* and *Arbuscular mycorrhizal* fungi in the soybean rhizosphere: a review. Plant Growth Reg. Accepted in press
Meena RS, Meena PD, Yadav GS, Yadav SS (2017b) Phosphate solubilizing microorganisms, principles and application of microphos technology. J Clean Prod 145:157–158
Mehdi Z, Nahid S-R, Alikhani HA, Nasser A (2006) Responses of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi. J Plant Nutr 29:1509–1522
Mehrvarz S, Chaichi MR, Alikhani HA (2008) Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barley (Hordeum vulgare L.). Am Eurasian J Agric Environ Sci 3:822–828

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial plant pathogenic and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663

Molla MN, Solaiman ARM (2009) Association of arbuscular mycorrhizal fungi with leguminous crops grown in different agro-ecological zones of Bangladesh. Arch Agron Soil Sci 55:233–245

Morel MA, Braña V, Castro-Sowinski S (2012) Legume crops, importance and use of bacterial inoculation to increase production. In: Goyal A (ed) Crop plant. ISBN: 978-953-51 0527-5, http://www.intechopen.com/books/crop-plant/legume-crops-importance-and-useofbacterial-inoculation-to-increase-production. Last visited on April, 2017

Mosse B (1977) Plant growth responses to vesicular-arbuscular mycorrhiza. X. Responses to Stylosanthes and maize to inoculation in unsterile soils. New Phytol 78:277–288

Mosse B, Powell CL, Hayman DS (1976) Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol 76:331–342

Motsara MR, Bhattacharyya P, Srivastava B (1995) Biofertilizer technology, marketing and usage-A source book-cum-glosarry. Fertilizer Development and Consultation Organisation, New Delhi

Muchovej RM (2001) Importance of mycorrhizae for agricultural crops. http://www.phc.eu/files/publicaties/Ag_Mycorrhizae_Public_document.pdf. Last accessed April 2017

Mugnier J, Jung G (1985) Survival of bacteria and fungi in relation to water activity and solvent properties of water in biopolymer gels. Appl Environ Microbiol 50:108–114

Muleta D (2010) Legume responses to arbuscular mycorrhizal fungi inoculation in sustainable agriculture. In: Khan MS et al (eds) Microbes for legume improvement. Springer, Wien. https://doi.org/10.1007/978-3-211-99753-6_12

Munchbach M, Nocker A, Narberhaus F (1999) Multiple small heat shock proteins in rhizobia. J Bacteriol 181:83–90

Munns DN, Mosse B (1980) Mineral nutrition of legume crops. In: Summerfield RJ, Bunting AH (eds) Advances in legume science. HMSO, London

Napamornbodi O, Rajanasiriwong W, Thamsurakul S (1988) Production of VAM fungi, *Glomus intraradices* and *G. mosseae* in tissue culture. In: Mycorrhiza for green Asia. University of Madras, Tamil Nadu

Navazio L, Moscatiello R, Genre A, Novero M, Baldan B, Bonfante P, Mariani P (2007) A diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. Plant Physiol 144:673–681

Nethravathi CS, Brahmaprakash GP (2005) Alginate based composite biofertilizer of *Bradyrhizobium japonicum* and *Bacillus megaterium* for soybean (*Glycine max* (L) Merrill). J Soil Biol Ecol 25:1–13

Nkaa FA, Nwokeocha OW, Ihuoma O (2014) Effect of phosphorus fertilizer on growth and yield of cowpea (*Vigna unguiculata*). J Pharm Biol Sci 9:74–82

Oldroyd GED, Harrison MJ, Udvardi M (2005) Peace talks and trade deals: keys to long-term harmony in legume-microbe symbioses. Plant Physiol 137:1205–1210

Olsen PE, Rice WA, Collins MM (1994a) Biological contaminants in North American legume inoculants. Soil Biol Biochem 27:699–701

Olsen PE, Rice WA, Bordeleau LM, Biederbeck VO (1994b) Analysis and regulation of legume inoculants in Canada: the need for an increase in standards. Plant Soil 161:127–134

Paau AS, Graham LL, Bennett M (1991) Progress in formulation research for PGPR and biocontrol inoculants. In: Keel C, Koller B, Défago G (eds) Plant growth-promoting rhizobacteria-progress and prospects. IOBC/WPRS Bulletin, Zurich

Pagano MC, Cabello MN, Scotti MR (2007) Phosphorus response of three native Brazilian trees to inoculation with four arbuscular mycorrhizal fungi. J Agric Technol 3:231–240

Pelczar MJ Jr, Chan ECS, Krieg NR (1993) Microbiology, 5th edn. Tata McGraw-Hill Publishing Company Limited, New Delhi

Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BIR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khans DF, Hauggaard-Nielsen H, Jensen BS (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48:1–17

Philip K, Jauhri KS (1984) Pressmud: a potential carrier for *Rhizobium* and *Azotobacter* 1. Comparative analytical studies of various carrier materials. Z Mikrobiol 139:5–4

Poinsot V, Bélanger E, Laberge S, Yang GP, Antoun H, Cloutier J, Treilhou M, Dénarié J, Promé JC, Debellé F (2001) Unusual methyl-branched a, ß-unsaturated acyl chain substitutions in the Nod factors of an Arctic *Rhizobium*, *Mesorhizobium* sp. Strain N33 (*Oxytropicarctobia*). J Bacteriol 183:3721–3728

Polonenko DR (1994) Commercial opportunities for multiorganism inoculants. In: Germida JJ (ed) Proceedings of the BIOREM 4th annual general meeting. BIOREM, Montreal

Postgate JR (1989) Trends and perspectives in nitrogen fixation research. Adv Microb Physiol 30:1–22

Raja N (2013) Biopesticides and biofertilizers: ecofriendly sources for sustainable agriculture. J Biofertil Biopestic. doi:1000e112:1000e112

Ram K, Meena RS (2014) Evaluation of pearl millet and mungbean intercropping systems in arid region of Rajasthan (India). Bangladesh J Bot 43(3):367–370

Reddy AA (2004) Consumption pattern, trade and production potential of pulses. Econ Polit Wkly 39:4854–4860. http://ssrn.com/abstract=1537541

Reddy AA (2009) Pulses production technology: status and way forward. Econ Polit Wkly 44:73–82

Reddy AA, Reddy GP (2010) Supply side constrains in production of pulses in India: a case study of lentil. Agric Econ Res Rev 23:129–136

Redecker D, Raab P (2006) Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia 98:885–895

Rewari RB (1984) Summarised results of microbiology trials. All-India Co-ordinated Research Project on Improvement of Pulses, New Delhi

Rewari RB (1985) Summarised results of microbiology trials. All-India Co-ordinated Research Project on Improvement of Pulses, New Delhi

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorous by plants. Aust J Plant Physiol 28(9):897–906

Rooge RB, Patil VC, Ravikrishnan (1998) Effect of phosphorus application with phosphate solubilisation organisms on the yield, quality and P uptake of soybean. Legum Res 21:85–90

Sadasivam KV, Tyagi RK, Ramarethinam S (1986) Evaluation of some agricultural wastes as carriers for bacterial inoculants. Agric Wastes 17:301–306

Sahgal M, Johri BN (2003) The changing face of rhizobial systematics. Curr Sci 84:43–48

Sahoo RK, Ansari MW, Pradhan M, Dangar TK, Mohanty S, Tuteja N (2014) Phenotypic and molecular characterization of efficient native Azospirillum strains from rice fields for crop improvement. Protoplasma. https://doi.org/10.1007/s00709-013-0607-7

Sanginga N (2003) Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. Plant Soil 252:25–39

Santos VB, Araujo SF, Leite LF, Nunes LA, Melo JW (2012) Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. Geoderma 170:227–231

Sattar MA, Khanam D, Ahmad S, Haider MR, Podder AK, Bhuiyan MA (1997) On-farm experiments on rhizobial inoculants in Bangladesh: results, problems, and possible solutions. In: Rupela OP, Johansen C, Herridge DF (eds) Extending nitrogen fixation research to farmers' fields. ICRISAT, Hyderabad

Saxena D, Mohammed A, Khanna S (1996) Modulation of protein profiles in *Rhizobium* sp under salt stress. Can J Microbiol 42:617–620

Saxena AK, Rathi SK, Tilak KVBR (1997) Differential effect of various endomycorrhizal fungi on nodulating ability of green gram by *Bradyrhizobium* sp. (*Vigna*) strain S24. Biol Fertil Soils 24:175–178

Schuessler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Shanker S, Brahmaprakash GP (2004) Development of composite biofertilizer containing *Bradyrhizobium+Bacillus megaterium* in different formulations. J Soil Biol 24:63–70

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2:587. http://www.springerplus.com/content/2/1/587

Siddiqui ZA, Baghel G, Akhtar MS (2007) Biocontrol of *Meloidogyne javanica* by *Rhizobium* and plant growth-promoting rhizobacteria on lentil. World J Microbiol Biotechnol 23:435–441

Silvester WB (1975) Ecological and economical significance of the non-legume symbioses. In: Newton WE, Nyman CJ (eds) 1st international symposium onnitrogen fixation. Washington State University Press, Washington, DC

Singh MS (2005) Effect of Rhizobium, FYM and chemical fertilizers on legume crops and nutrient status of soil-a review. Agric Rev 26:309–312

Singh CS, Amawate JS, Tyagi SP, Kapoor A (1990) Interaction effect of *Glomus fasciculatum* and *Azospirillum brasilense* on yields of various genotypes of wheat (*Triticum aestivum*) in pots. Zentralbl Mikrobiol 145:203–208

Singh CS, Kapoor A, Wange SS (1991) The enhancement of root colonisation of legumes by vesicular-arbuscular mycorrhizal (VAM) fungi through the inoculation of the legume seed with commercial yeast (*Saccharomyces cerevisiae*). Plant Soil 131:129–133

Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Singleton PW, Boonkerd N, Carr TJ, Thompson JA (1997) Technical and market constraints limiting legume inoculant use in Asia. In: Rupela OP, Johansen C, Herridge DF (eds) Extending nitrogen fixation researchto farmers' fields. ICRISAT, Hyderabad

Singleton P, Keyser H, Sande E (2002) Development and evaluation of liquid inoculants. In: Herridge D (ed) Inoculants and nitrogen fixation of legume in Vietnam. ACIAR Proceedings

Sinha RK, Valani D, Chauhan K, Agarwal S (2014) Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: reviving the dreams of Sir Charles Darwin. Int J Agric Health Saf 1:50–64

Slattery JJ, Coventry DR, Slattery W (2001) Rhizobial ecology as affected by the soil environment. Aust J Exp Agric 41:289–298

Smidsrod O, Skjak-Braek G (1990) Alginate as immobilization matrix for cells. Trends Biotechnol 8:71–78

Smith RS (1992) Legume inoculant formulation and application. Can J Microbiol 38:485–492

Smith RS (1995) Inoculant formulations and applications to meet changing needs. In: Tikhonovich IA, Provorov NA, Romanov VI, Newton WE (eds) Nitrogen fixation: fundamentals and applications. Kluwer Academic Publishers, Dordrecht

Smith SE, Read DJ, Harley JL (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Sougoufara B, Diem HG, Dommergues YR (1989) Response of field -grown *Casuarina equisetifolia* to inoculation with *Frankia* strain ORS 021001 entrapped in alginate beads. Plant Soil 118:133–137

Sparrow SD, Ham GE (1983) Survival of *Rhizobium phaseoli* in six carrier materials. J Agron 75:181–184

Sprent JI (2001) Nodulation in legumes. Royal Botanic Gardens, London

Sridhar V, Brahmaprakash GP, Hegde SV (2004) Development of a liquid inoculant using osmoprotectants for phosphate solubilizing bacteria. Karnataka J Agric Sci 17:251–257

Stancheva I, Geneva M, Djonova E, Kaloyanova N, Sichanova M, Boychinova M, Georgiev G (2008) Response of alfalfa (*Medicago sativa* L) growth at low accessible phosphorus source to the dual inoculation with mycorrhizal fungi and nitrogen fixing bacteria. Gen Appl Plant Physiol 34:319–326

Stanier RY (1987) General microbiology, 5th edn. Macmillan, London

Streeter JG (1985) Accumulation of *alpha*, *alpha*-trehalose by *Rhizobium* bacteria and bacteroids. J Bacteriol 164:78–84

Subba Rao NS, Tilak KVBR (1977) Souvenir bulletin. Directorate of Pulses Development, Government of India

Subba Rao NS, Tilak KVBR, Singh CS (1986) Dual inoculation with *Rhizobium* sp. and *Glomus fasciculatum* enhances nodulation, yield and nitrogen fixation in chickpea (*Cicer arietinum* Linn). Plant Soil 95:351–359

Suja G (2008) Strategies for organic production of tropical tuber crops. In: Venkateswarlu B, Balloli SS, Ramakrishna YS (eds) Organic farming in rain fed agriculture: opportunities and constraints. Central Research Institute for Dryland Agriculture, Hyderabad, pp 135–143

Surendra ST, Pathan MA, Gupta KP, Khandkar UR (1993) Effect of phosphate solubilising bacteria at different levels of phosphate on black gram. India J Agron 38:131–133

Tagore GS, Namdeo SL, Sharma SK, Kumar N (2013) Effect of *Rhizobium* and phosphate solubilizing bacterial inoculants on symbiotic traits, nodule leghemoglobin, and yield of chickpea genotypes. Int J Agron 2013:1–8. Article ID 581627, 8 pages. https://doi.org/10.1155/2013/581627

Thies JE, Woomer PL, Singleton PW (1995) Enrichment of *Bradyrhizobium* spp. populations in soil due to cropping of the homologous host legume. Soil Biol Biochem 27:633–637

Tittabutr P, Payakapong W, Teaumroong N, Singleton PW, Boonkerd N (2007) Growth, survival and field performance of bradyrhizobial liquid inoculants formulations with polymeric additives. Sci Asia 33:69–77

Trivedi P, Pandey A, Palni LMS (2012) Bacterial inoculants for field applications under mountain ecosystem: present initiatives and future prospects. In: Maheshwari DK (ed) Bacteria in agrobiology: plant probiotics. Springer, Berlin. https://doi.org/10.1007/978-3-642-27515-9_2

Valsalakumar N, Ray JG, Potty VP (2007) Arbuscular mycorrhizal fungi associated with green gram in south India. Agron J 99:1260–1264

van der Heijden MGA, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A, Sanders IR (2006) The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytol 172:739–752

van der Heijden MGA, Rinaudo V, Verbruggen E, Scherrer C, Bàrberi P, Giovannetti M (2008) The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. In: 16th IFOAM organic world congress, Modena

Van Elsas JD, Heijnen CE (1990) Methods for the introduction of bacteria into soil: a review. Biol Fertil Soils 10:127–133

Vankessel C, Singleton PW, Hoben HJ (1985) Enhanced N-transfer from a soybean to maize by vesicular arbuscular mycorrhizal (VAM) fungi. Plant Physiol 79:562–563

Varma D, Meena RS (2016) Mungbean yield and nutrient uptake performance in response of NPK and lime levels under acid soil in Vindhyan region, India. J App Nat Sci 8(2):860–863

Varma D, Meena RS, Kumar S (2017) Response of mungbean to fertility and lime levels under soil acidity in an alley cropping system in Vindhyan Region, India. Int J Chem Stud 5(2):384–389

Vejan P, Abdullah R, Khadiran T, Ismail S, Boyce AN (2016) Review role of plant growth promoting Rhizobacteria in agricultural sustainability—a review. Molecules 21:573. https://doi.org/10.3390/molecules21050573

Velineni S, Brahmaprakash GP (2011) Survival and phosphate solubilizing ability of *Bacillus megaterium* in liquid inoculants under high temperature and desiccation stress. J Agric Sci Technol 13:795–802

Venkataraman GS (1981) Blue green algae for rice production—a manual for its production'. F.A.O. Soil Bulletin. 46, p 102

Verma JP, Meena VS, Kumar A, Meena RS (2015a) Issues and challenges about sustainable agriculture production for management of natural resources to sustain soil fertility and health: a book review. J Clean Prod 107:793–794

Verma JP, Jaiswal DK, Meena VS, Meena RS (2015b) Current need of organic farming for enhancing sustainable agriculture. J Clean Prod 102:545–547

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vincent JM, Thompson JA, Donovan KO (1962) Death of root nodule bacteria on drying. Aust J Agric Res 13:258

Vithal N (2004) Development of liquid inoculant formulations for *Bradyrhizobium* sp.: (*Arachis*), *Azospirillum lipoferum* and *Azotobacter chroococcum*. Ph.D. thesis, University of Agricultural Sciences, Bangalore

Walter JF, Paau AS (1993) Microbial inoculant production and formulation. In: Metting FB Jr (ed) Soil microbial ecology. Marcel Dekker, New York

Wani SP, Lee KK (1991) Role of biofertilizers in upland crop production. In: Tandon HLS (ed) Fertilizers of organic manures, recycle waste and biofertilizers. Fertilizer Development and Consultation Organization, New Delhi

Wani SP, Lee KK (2002) Biofertilizers for sustaining cereal crop production. In: Kannaiyan S (ed) Biotechnology of biofertilizers. Narosa publishing House, New Delhi

Wani SP, Rupela OP, Lee KK (1995) Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. Plant Soil 174:29–49

Weber E, George E, Beck DP, Saxena MC, Marschner H (1992) Vesicular-arbuscular mycorrhiza and phosphorus uptake of chickpea grown in Northern Syria. Exp Agric 28(433):442

Xavier LJC, Germida JJ (2002) Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy. Soil Biol Biochem 34:181–188

Xavier IJ, Holloway G, Leggett M (2004) Development of rhizobial inoculant formulations. In: Proceedings of the great plains inoculant forum. Plant Management Network, Saskatoon, Saskatchewan

Yadav GS, Babu S, Meena RS, Debnath C, Saha P, Debbaram C, Datta M (2017) Effects of godawariphosgold and single supper phosphate on groundnut (*Arachis hypogaea*) productivity, phosphorus uptake, phosphorus use efficiency and economics. Indian J Agric Sci 87(9):1165–1169

Youssef MMA, Eissa MFM (2014) Biofertilizers and their role in management of plant parasitic nematodes – a review. J Biotechnol Pharm Res 5:1–6

Zaidi A, Khan MS, Amil M (2003) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Eur J Agron 19:15–21

Zarei M, Saleh-Rastin N, Alikhani HA, Aliasgharzadeh N (2006) Responses of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi. J Plant Nutr 29:1509–1522

View publication stats